



INDIAN AGRICULTURAL
RESEARCH INSTITUTE, NEW DELHI.

L. A. R. I. G.

MGIPC—88—45 AR/52—8-6-53—1,000.

PHILOSOPHICAL TRANSACTIONS

OF THE

ROYAL SOCIETY OF LONDON

SERIES B

VOLUME 226

BIOLOGICAL SCIENCES

LONDON

Printed and published for the Royal Society
By Harrison & Sons, Ltd., 44-47, St. Martin's Lane

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THE Committee appointed by the *Royal Society* to direct the publication of the *Philosophical Transactions* take this opportunity to acquaint the public that it fully appears, as well from the Council-books and Journals of the Society as from repeated declarations which have been made in several former *Transactions*, that the printing of them was always, from time to time, the single act of the respective Secretaries, till the Forty-seventh Volume ; the Society, as a Body, never interesting themselves any further in their publication than by occasionally recommending the revival of them to some of their Secretaries, when, from the particular circumstances of their affairs, the *Transactions* had happened for any length of time to be intermitted. And this seems principally to have been done with a view to satisfy the public that their usual meetings were then continued, for the improvement of knowledge and benefit of mankind : the great ends of their first institution by the Royal Charters, and which they have ever since steadily pursued.

But the Society being of late years greatly enlarged, and their communications more numerous, it was thought advisable that a Committee of their members should be appointed to reconsider the papers read before them, and select out of them such as they should judge most proper for publication in the future *Transactions* ; which was accordingly done upon the 26th of March, 1752. And the grounds of their choice are, and will continue to be, the importance and singularity of the subjects, or the advantageous manner of treating them : without pretending to answer for the certainty of the facts, or propriety of the reasonings contained in the several papers so published, which must still rest on the credit or judgment of their respective authors.

It is likewise necessary on this occasion to remark, that it is an established rule of the Society, to which they will always adhere, never to give their opinion, as a Body upon any subject, either of Nature or Art, that comes before them. And therefore the thanks, which are frequently proposed from the Chair, to be given to the authors of such papers as are read at their accustomed meetings, or to the persons through whose hands they received them, are to be considered in no other light than as a matter of civility, in return for the respect shown to the Society by those communications. The like also is to be said with regard to the several projects, inventions, and curiosities of various kinds, which are often exhibited to the Society ; the authors whereof, or those who exhibit them, frequently take the liberty to report, and even to certify in the public newspapers, that they have met with the highest applause and approbation. And therefore it is hoped that no regard will hereafter be paid to such reports and public notices ; which in some instances have been too lightly credited, to the dishonour of the Society.

I—On the Structure of the Skull in the Mammal-like Reptiles of the Suborder Therocephalia

By R. BROOM, D.Sc., F.R.S.

Transvaal Museum, Pretoria, S. Africa

(Received April 29, 1935)

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I—INTRODUCTION

The first-known mammal-like reptiles were discovered by ANDREW GEDDES BAIN (1845) in the Karroo Beds of South Africa about a hundred years ago. The large majority of the species he discovered belong to the Anomodont group, of which *Dicynodon* is the best-known genus—characterized by having a tortoise-like beak with or without permanent-growing, large, upper canines. Carnivorous types are very much rarer than the vegetarian Anomodonts, and BAIN was successful in getting only comparatively few specimens, and most of these in a very unsatisfactory condition. Most of his collecting was done in what we now regard as the middle zones of the Karroo, and the majority of his specimens belong to the suborders Gorgonopsia and Cynodontia.

SEELEY in 1889 described an imperfect skull under the name *Hyorhynchus platyceps*; but as the teeth were lost and the skull much weathered, the affinities of the form

were not very manifest. In 1894 SEELEY described the point of the snout of an allied form which was characterized by having six incisors in each premaxilla. He further greatly added to our knowledge by describing some good skulls of the later carnivorous mammal-like reptiles belonging to the suborder Cynodontia, and to the suborder we now call the Bauriamorpha; but until about thirty years ago very little was known of the earlier carnivorous types.

In 1902 I found in the collection of the South African Museum, Capetown, the anterior half of the skull of a "Theriodont" from the Lower Karroo Beds, in which the palate proved on development to be quite unlike that of the higher "Theriodonts" such as *Cynognathus* and *Gomphognathus* described by SEELEY, and to be essentially similar in type to that of the Rhynchocephalians. I therefore proposed to place the primitive "Theriodonts" in an order or suborder by themselves, the Therocephalia.

During the last thirty years a large number of Therocephalian genera and species have been described from the Lower Karroo Beds, which form the *Tapinocephalus* zone. Only very few small Therocephalians are known from the base to the top of the *Endothiodon* zone; but in the upper beds of the *Cistecephalus* zone a considerable number of fairly large Therocephalians has been found. In the *Lystrosaurus* zone numbers of small Therocephalians are found which connect the typical Therocephalians with the Bauriamorphs of the *Cynognathus* zone. Of these small forms, *Scaloposaurus* and *Ericiolacerta* are the best known. In my recent book (1932) on the "Mammal-Like Reptiles of South Africa", I have retained them among the Therocephalians; while WATSON (1931) has, in a recent paper, placed them among the Bauriamorphs. We certainly have a series of types such as *Ictidognathus*, *Icticephalus*, *Choerosaurus*, *Scaloposaurus*, *Ericiolacerta*—all small mammal-like reptiles where the earlier forms have a typical Therocephalian palate, and the later forms have added a secondary palate, and thus approach closely to the Bauriamorphs proper. It matters little at present where we draw the dividing line.

In the present paper I wish to deal mainly with the primitive Therocephalians, as these are amongst the least satisfactorily known of the mammal-like reptiles. It may seem strange to speak of the group as imperfectly known, considering that in my recent book I recognized 25 genera and 30 species, all known by skulls or snouts, and many by nearly perfect skulls. But though many skulls have been described we know little of the anatomy except the relations of the external bones. Something is known of the palate in half a dozen genera, and a little of the structure of the lower jaw, but little of the occiput or of the structure of the brain-case, and very little of the dental succession. Our ignorance of the earlier Therocephalian skull is largely due to the fact that nearly every skull from the *Tapinocephalus* zone is in a matrix so hard that satisfactory development is very difficult, and in many cases the bones are cracked so that the tracing of sutures is hardly possible.

The Therocephalians of the *Tapinocephalus* zone can conveniently be placed in two groups or families—those with two large functional upper canines, and those with only one. In mammals with a full dentition the canine may be defined as the first maxillary tooth; but in many of the Therocephalians this is not so. Very frequently

the first maxillary tooth is quite small, and it is the second tooth that is the killing tooth. Occasionally there are two small maxillary teeth in front of the large supposed canine. One might be inclined to support the view that the first maxillary tooth, even if small, is the true canine, and that the supposed canine is a molar or premolar. But it is not improbable that the canine of the Cynodont and the mammal is the homologue of the third maxillary tooth of those early Therocephalians which have apparently three canines, and that the one or two small anterior maxillary teeth of the lower Therocephalians are completely lost in later forms.

There has been a similar difficulty with those Therocephalians in which there appear to be two large canines. When the first skulls were discovered with two large canines the question naturally arose as to whether they might not be skulls at a stage in development corresponding to a half-grown cat or dog where a deciduous and a permanent canine might both for a time be functional. The type-skull of the Cynodont *Trirachodon Kannemeyeri* has two functioning canines, one of the first and one of the second set. But too many skulls of early Therocephalians have been found with two large canines to admit of the possibility of their belonging to different sets.

To get some light on the dental succession, and to get to know fully the structures of the brain-case, I resolved to have a number of skulls or portions of skulls cut in sections. There was, of course, the difficulty that Therocephalian skulls are rare, and the large majority are types which one would hesitate to damage by having sliced. Fortunately, I had collected at various times quite a number of specimens which were not good enough to be regarded as museum specimens—either badly weathered, or having lost the snouts indeterminate, or specimens where the teeth had been lost and where there was a danger of some thoughtless person making a type of a skull without teeth. I have therefore, with the aid of a grant from the Royal Society, had a number of specimens which are of little museum value cut in slices, and have been able to study most details of the internal structure. Unfortunately, the majority of the specimens have had the outer bones much weathered, and in these it is difficult to trace the outer sutures.

Since the present investigation was started, DR. L. D. BOONSTRA, of the South African Museum, Cape Town, has published two papers (1934, 1935) on Therocephalians and four on Gorgonopsians. The earlier Therocephalian paper deals mainly with specimens which I had collected, but concerning which, being steadily engaged in medical practice, I had no opportunity of doing more than giving preliminary descriptions, and which specimens I had disposed of to the British Museum. These specimens, and one or two others in the British Museum, DR. BOONSTRA has developed much further, and he has revealed a number of details of the inner structure not previously known. The papers are illustrated by a number of good drawings by Mrs. BOONSTRA; but the other drawings are apparently by BOONSTRA himself. Some of the latter are extremely diagrammatic, and others are so carelessly and inaccurately drawn that one is in doubt as to what reliance can be placed on any of them. For example, he gives a side-view of the skull of *Scylacosaurus sclateri* (B.M. R4055) in which he figures 8 molars, but in the palatal view of the same skull,

apparently drawn by Mrs. BOONSTRA, there are only 7 molars, and in the letterpress BOONSTRA states that he believes there are only 7. In his side-view of the skull *Arctognathoides breviceps* (S.A.M. 9345) he figures 5 large incisors, but in his palatal view of the same skull he figures 4 extremely minute incisors. In the same figure he shows 6 very minute molars while in the letterpress he says "the molars are large".

WATSON, in 1921, had published figures of part of the brain-case in the Gorgonopsians *Scymnognathus whaitsi* and *Leptotrachelus eupachygnathus* showing the anterior process from the prootic [the pleurospenoid]. These figures of WATSON's BOONSTRA has reproduced with little modification. WATSON also showed a similar condition in the Therocephalian *Scymnosaurus watsoni*. BOONSTRA has shown a somewhat similar condition in the Therocephalians *Whaitsia major*, *Notosollasia laticeps*, and *Trochosaurus major*. But his figures are extremely diagrammatic, and appear to be inaccurate in many details. In one of his figures he shows the fifth nerve passing through what he believes to be the prootic.

BOONSTRA's later paper (1935) is on some Therocephalian skulls in the Broom collection in the American Museum. The paper deals mainly with questions of taxonomy.

II—TRANSVERSE SECTIONS OF THE SNOUT OF *Trochosaurus dirus*, sp. nov.

The only specimen I have had cut which shows satisfactorily the front of the snout is the anterior part of the skull of a large Therocephalian which, after it was cut, was seen to belong to a new species. In each premaxilla there are 5 large pointed incisors, and there are two large functional canines. In the lower jaw there are 3 incisors, 1 canine, and 3 molars. The form is manifestly a near ally of *Trochosaurus major*, though very much larger. The type species has 4 upper molars, and possibly this new species may prove to have only 2. It is thus possible that it belongs to a new genus; but one hesitates to establish a new genus on a snout which does not show the upper molars, and little harm can be done by provisionally leaving the form in the genus *Trochosaurus*. The 5 upper incisors measure in length 57 mm, as compared with 45 mm in *Trochosaurus major*. The first canine has an antero-posterior length of 25 mm and a width of 10 mm. The 3 lower molars together measure 23 mm. The snout in the anterior canine region has a width of about 85 mm.

The specimen has been cut first horizontally near the bases of the incisor teeth, and then the upper portion has been cut into five pieces by four transverse cuts. The slabs vary in thickness from about 13.5 mm to about 18.5 mm; and each slice has removed about 2 mm of the specimen. We are thus able to study eight transverse sections of the snout, of which 1 and 2, 3 and 4, 5 and 6, 7 and 8 are each about 2 mm apart and each pair about 15 mm from the next pair. In the figures I give of the sections, sections 1, 3, 5, and 7 have been drawn reversed, so that each section is as it would appear if viewed from the front. And in addition to these, there is the basal section, and a second nearly complete basal section 2 mm higher up. This second differs so little from the other that it need not be considered.

Basal section—As will be seen from the figure given, fig 1, Plate 1, this section shows all the ten upper incisors cut across, the peculiar arrangement of the upper canines, and the anterior teeth of the lower jaws cut across. On the left side the 1st, 2nd, and 4th incisors are seen to be well-developed teeth with the pulp cavities completely or nearly filled with dentine; but the 5th incisor is evidently a younger tooth, as its pulp cavity is still fairly large, while the 3rd incisor is evidently quite a young tooth apparently just recently through the gum. On the right side the 1st, 2nd, and 3rd incisors are all old teeth, and the 5th has the pulp cavity nearly filled with dentine. The 4th incisor has been shed and a new tooth, of which the top only appears in the section, is taking its place. The canine condition is very remarkable. On the left side a large functional canine is seen cut across, with a fairly large pulp cavity. This is clearly the principal canine. On its inner side there is seen cut across part of a very young replacing canine. Behind these two canines are two others unfortunately badly preserved, and of these it is the inner one which is functional, the outer one being replaced. On the right side the specimen is very imperfect. There is a large functional anterior canine, and on its inner side is seen the tip of the replacing anterior canine. Nothing is seen of the posterior canines in the section, but the upper part of the specimen reveals much of a large young, but apparently functioning, canine which clearly corresponds to the inner of the two posterior canines seen on the left side. The nature of the dental succession will be further considered when the transverse sections have been studied.

Three lower incisors are seen on the left side, the 3rd being only the tip of the quite young tooth. There is only a single canine, which is very much smaller than the anterior upper canine. On the right side the 1st incisor is represented by only the tip of a young tooth, and the 3rd is not seen. Doubtless a 3rd incisor has just been shed, and the replacing one has not yet reached the level of the section. No molars are seen in the section, but three are well preserved on the base of the specimen.

Transverse section 1, fig. 2, Plate 1. This section in the middle line is about 19 mm behind the front of the snout. It is slightly oblique. On the left side the functional i^2 is seen cut obliquely across. On its inner side is seen a small part of the cavity in which the successional i^1 is developing. On the outer side of i^2 is part of the young i^3 seen in the horizontal section. On the right side the condition a little farther back is seen. The second incisor is seen cut higher up, and a little concavity on its inner side is due to absorption by the developing succeeding i^2 . The developing successional i^1 is seen on the inner side of i^2 and outside i^2 is seen i^3 cut across. Above the premaxillaries on each side, portions of the anterior ends of the septomaxillaries are seen, though the ascending internasal processes of the premaxillaries are not preserved in the specimen. I have figured where they would be, had they been preserved. The bases are seen on the top of this first slab.

Transverse section 2, fig. 3, Plate 1. This section, which is about 2 mm behind section 1, shows for the most part the same teeth as seen in the other. As it seems probable that there are more than two sets of teeth, we cannot refer to the one as the deciduous set and to the other as the permanent, but it will be convenient to refer to

the older set as A, and the other as set B. Near the middle line are seen the developing first incisors of set B. On the right side the root of Ai^3 is seen considerably absorbed by the developing Bi^2 . Above each premaxillary a considerable part of the septomaxillary is seen in section.

Transverse section 3, fig. 4, Plate 1. This section is 13.5 mm behind section 2; and is very different in appearance. Immediately behind the anterior powerful part of the premaxillaries that support the roots of the front incisors the anterior part of the nasal cavity forms a deep depression whose floor is formed by palatal plates of the premaxillaries. Above these plates are seen near the middle line the anterior ends of the prevomers. Above the upper parts of the premaxillaries are seen sections of the septomaxillaries. The teeth seen on the left side are part of Ai^4 , and a section of the developing Bi^3 with a portion of the root of Ai^3 . On the right side near the upper part of the premaxilla is seen a section of the root of Ai^3 and on its inner side the developing Bi^3 . In the lower and outer part of the premaxilla is a complete section of the developing Bi^4 with portions of the root of Ai^4 .

Transverse section 4, fig. 5, Plate 1. This section is about 2 mm behind section 3. The teeth shown are practically the same as in the previous section. Ai^3 , on the right side, is here only represented by the tip of the root.

Transverse section 5, fig. 6, Plate 1. This section is about 15 mm behind the previous one. Here we have only the posterior parts of the premaxillaries seen, but have the anterior part of one maxilla, and a section of each nasal and septomaxilla. The bones of the prevomers are considerably fractured, and it is impossible to say whether all these median bony fragments are part of the prevomers or in part premaxillary. From the condition seen in the next snout to be described, most probably the lower portions are parts of the premaxillaries. The outer fragment on the left side probably is. On the left side of the section is seen a section across the 5th upper incisor apparently of the A set. There is no evidence of any succeeding tooth. On the right side the section passes behind the 5th incisor. On each side is seen a section of one of the lower canines, showing the position of these teeth in natural occlusion.

Transverse section 6, fig. 7, Plate 1. This section is about 2 mm behind section 5, and it is essentially similar, but here on both sides the nasal meets the septomaxillary. On the left side the root of the 5th incisor is seen, but, as in the other section, there is no evidence of a succeeding tooth germ.

Transverse section 7, fig. 8, Plate 1. This section is from 17 to 19 mm behind section 6. Here the nasals are seen as large, thick bones. From the fact that the surface of the nasals is grooved somewhat like the surface of the horn core of an ungulate apparently by blood vessels one may infer that over the nasals there was in life a thick, horny plate or plates. In the closely allied *Lycosuchus* the nasals are united, and there was probably a considerable horny boss developed on the snout. The section passes through the posterior ends of the septomaxillaries. Below the outer sutures of the nasals are seen on either side small bony fragments. These are possibly, but not certainly, detached portions of the nasals. There has been a little crushing of the specimen, and parts of the maxillae are seen to be fractured. The prevomers are well

developed. On the left side is seen a section of the large anterior canine. On the right side is a section of the canine with, on its inner side, a developing succeeding canine.

Transverse section 8, fig. 9, Plate 1. This section is 2 mm behind section 7. Here, inside of the canine on the left side, is seen part of the very young succeeding canine. On the right side the large outer canine is seen, with the young succeeding tooth alongside of it.

The sections of the snout of *Trochosaurus dirus* show clearly the general structure and relations of the premaxillary, maxillary, septomaxillary, and nasal bones, and in part the relations of the prevomers. We also have considerable light thrown on the dental succession.

There are five incisors, and certainly replacing teeth developing in connexion with the first four. The five incisors are probably not all of one set, for some are clearly older than others. Thus i^3 on the left side is a young tooth not fully developed, and it has replaced an earlier i^3 , of which remains are seen. And on the right side a 4th incisor has recently been shed and a new tooth is developing in its place. It seems probable that there is a continuous succession of incisors.

The canine condition is of great interest. There are two large functional canines which are placed so close together that they probably function as one. This has long been known in a number of genera and species. We now know that each has a replacing tooth. In the specimen studied each anterior canine has a very young replacing tooth; but the posterior canine on the left side is being replaced by an already well-developed successor. On the right side the specimen is imperfect, but the inner canine is of large size and apparently functional. Probably the outer posterior canine is shed or being absorbed.

We have no evidence in the specimen of the upper molars. In the lower jaw there is clear evidence that there is a dental succession in the incisors. There is only a single canine in each mandible, and though there is no evidence of a dental succession, not improbably this canine is replaced from time to time. There are three lower molars, and the specimen shows no evidence of any succession in these.

III—TRANSVERSE SECTIONS OF THE SNOUT OF A MODERATE-SIZED THEROCEPHALIAN—PROBABLY *Pristerognathus vanderbyli*, BROOM

This snout has unfortunately the outer bones very badly weathered; but as it belongs to the group of Therocephalians which have only one large functional canine, and as it shows the greater part of the prevomers in perfect condition, it seems well worthy of being described. The slabs into which the specimen has been cut are from 5 to 7 mm in thickness, and the wheel has probably removed about 2 mm with each cut, so that each even-numbered section is about 5 to 7 mm behind the odd numbered in front, and 2 mm in front of the odd numbered behind it. Each even-numbered section has been figured in the reversed position to facilitate comparison with the odd numbered.

Section 1, fig. 10, Plate 1. This section is through the premaxillaries some distance behind the part of the bones which hold the front incisors, and across the anterior part of the nasal cavity. A part of the 4th upper incisor is seen in section. Portions of the septomaxilla are seen above the right premaxilla. In the nasal cavity the anterior end of the right prevomer is seen above the palatal plate of the premaxilla. Parts of the lower jaws are seen, with sections of what are apparently the 2nd and 3rd lower incisors.

Section 2, fig. 11, Plate 1. This section is very similar to the preceding one. Parts of both prevomers are here seen.

Section 3, fig. 12, Plate 1. This section still shows the palatal plates of the premaxillaries. Above them are broad plates of the prevomers. The nasals are somewhat crushed down, and the exact relations of the septomaxillary are not clearly shown. In the lower jaw the right canine is seen in section. There is no evidence of a replacing canine. It is interesting to note that the symphysis is rather loose, and even as far back as the canine region there is no evidence of the splenial.

Section 4, fig. 13, Plate 1. In this section the pair of large prevomers are seen curved for the support of a pair of large organs of Jacobson. Below the prevomers are a pair of rather stout palatine processes of the premaxillae. In the lower jaw we still have a section of the large canine, with no evidence of a replacing tooth.

Section 5, fig. 14, Plate 1. Here the prevomers are still large. The palatine processes of the premaxillae are cut near their posterior ends. Part of the right upper canine is seen in section. In the lower jaw part of the left canine is seen in section, and on the right side a molar tooth apparently molar 1. There is no evidence of a replacing tooth in connexion with either of these functional teeth. The anterior ends of the splenials are seen.

Section 6, fig. 15, Plate 1. This section much resembles the preceding one. The rather oblique right upper canine is partly seen, and there is still no evidence of a replacing canine. In the lower jaw we see on the left side part of the canine, with the root of what is probably the 1st molar. On the right side we see a molar tooth cut in section—probably the second. There is no evidence of any replacing teeth.

Sections 7 and 8, figs. 16 and 17, Plate 1. These sections much resemble section 6. The maxillae, each of which has part of the canine, are peculiarly shaped, approaching each other in the middle line, and having above them a wide expansion of the nasal cavity. In each section of the lower jaw molar teeth are seen in section, but there is not the faintest evidence of any replacing teeth.

Section 9, fig. 18, Plate 1. Here the prevomers are becoming narrower owing to the transverse ledge which supported Jacobson's organ disappearing. Each maxilla is largely occupied by the canine. In each mandible parts of three molars are seen in section. There is no evidence of any succeeding teeth.

Section 10, fig. 19, Plate 2. This section is very similar to section 9.

Section 11, fig. 20, Plate 2. Here are seen in the maxilla of the right side not only a section of the large canine but below it a section of a second tooth smaller than the large canine, but much larger than any of the molars. I regard it as the root of a small

second canine once functional, but now represented by the root only which is not yet absorbed. Below this second canine root is part of one of the molars—probably the 1st molar, but possibly the 2nd. The ridge of the maxilla that passes inwards is well developed, and just possibly the inner part is part of the palatine. There is a suture or crack as indicated in the figure, but I incline to regard it as a crack. On the left side there is below the inner part of the maxilla what might readily be regarded as a small upper molar. This is really the tip of one of the lower molars. Small parts of the dentaries and splenials are seen in the sections of the lower jaws.

Section 12, fig. 21, Plate 2. This section is fairly similar to the last, but here we have, on the right side at least, certainly a section of the anterior end of the palatine. On the left side there is above the inner ridge of the maxilla a small bony element which is possibly a part of the left palatine. In the lower part of the left maxilla is to be seen the tip of what is possibly a developing molar. This is the only evidence we have in any of the sections of a developing tooth, and it is not conclusive. Probably the replacing molars if they occur are developed, as in the crocodile, below the functional teeth. On the right side the tip of the root of the 2nd canine is seen below the upper end of the 1st canine. In the lower part of the maxilla a portion of one of the molars is seen. Part of one of the lower molars is seen on the right side.

Section 13, fig. 22, Plate 2. This section is near the posterior end of the free portion of the prevomers. On each side is seen a section of the palatines closely sutured to the lower parts of the maxillae. On the left side are seen sections of the roots of the 1st and 2nd canines ; and on the right side is a section of a root of a molar.

Section 14, fig. 23, Plate 2. This section, which like all the others is a little oblique, is on the right side just behind the internal nares, and on the left side just in front of the posterior end of the nasal opening. On the right side the palatine is seen sutured to the lower part of the maxilla and passing inwards as a thick bone which lies above the prevomer. On the left side part of the palatine is seen above the prevomer, and part sutured to the maxilla. The prevomers are no longer narrow, vertical bones, but broad and thick.

Section 15, fig. 24, Plate 2. This section is only a short distance behind the preceding one. The prevomers are still large and broad, and they are completely roofed over by the palatines, which are developed to form a powerful palate. This is the last satisfactory section cut from the specimen.

IV—TRANSVERSE SECTIONS OF THE SKULL OF *Pristerognathus minor* (HAUGHTON)

This skull was badly weathered and had lost the front of the snout, both jugal arches, the whole of the occiput, and the base of the brain case. It seemed likely, however, that sections would show the structure of the anterior two-thirds of the palate ; and this has proved to be the case.

The skull as preserved measured 195 mm in length, and probably measured when complete about 250 mm. By transverse cuts it was divided into 29 slabs. Each

slice is about 3.5 mm in thickness, and each cut has removed 3 mm of the specimen. We thus have 57 sections which we can study. Unfortunately, the bones on the outside of the specimen are badly weathered, and very few of the outer sutures can be traced. The centre of the specimen is extremely hard, and the whole of the structure of the palate from near the front of the prevomers to near the posterior end of the pterygoids can be satisfactorily made out. As the base of the brain case is lost the posterior sections need not be considered, as transverse sections of the brain region are much more satisfactorily seen in the next specimens to be studied.

Of the sections of the snout it seems unnecessary to figure and describe more than about half, as many are very similar to the adjoining sections. As the snout is broken off a little in front of the upper canines the first section we have, fig. 25, Plate 2, shows no part of the premaxillaries. The prevomers have broad basal portions and slender, ascending plates. The section is through the lower canines.

Section 3, fig. 26, Plate 2, is a little better preserved than section 1. The basal portion of the prevomers probably here supported a small organ of Jacobson. On the left side the section passes through part of the upper canine. On the inner side of the left maxilla the tip of the palatine is seen. In the lower jaws a molar tooth is seen in section on each side.

The next section figured is section 10, fig. 27, Plate 2. This is probably in the region of the 2nd upper molar, and it is probably this tooth of which a part is seen in section on the left side. The prevomers have very slender ascending plates, partly crushed in the specimen, and rather more strongly developed lower portions. There are here no ledges which could have supported Jacobson's organs. On the left side part of the palatine is seen, but the bones are weathered and the sutures are indistinct. Lower molars are seen in section on each side.

Section 12, fig. 28, Plate 2, is fairly similar to section 10, but more of the palatines are seen. The prevomers are here still more flattened.

Section 13, fig. 29, Plate 2, shows the lower part of each prevomer broadening out as it nears the posterior part of the internal nares. Sections of the palatines are seen on each side, but the bones are somewhat broken. In each maxilla and in each dentary a molar tooth is seen in section, and there is no clear evidence of succeeding teeth in connexion with any of them. On the right side the base of one molar is seen in the dentary, and the upper part of the molar which is in front of it.

Section 14, fig. 30, Plate 2, is just behind the posterior end of the internal nasal opening on the left side, and just in front of it on the right. On the left side the palatine extends from the maxilla to the middle line, the inner part lying above the prevomer. On the right side the outer part of the palatine is seen in connexion with the maxilla, and the inner end above the prevomer. The prevomers have lost the upper thin plates, and the lower parts are relatively broad. The section is essentially similar to section 14 of the last specimen.

Section 15, fig. 31, Plate 2, is a short distance behind the internal nares. On each side sections of tooth bearing portions of the maxillae are seen, each with part of the root of a molar. The palatines and prevomers form a complete palate. The

prevomers are here very wide, and they form the greater part of the roof of the mouth.

Section 16, fig. 32, Plate 2, is essentially similar to section 15. Though in both sections the palatines are not quite in contact with the maxillae on the right side this is evidently due to crushing.

Section 17, fig. 33, Plate 3. About the region of this section the palate begins to bend down rather abruptly. The prevomers are still fairly large and wide. The palatines appear more robust, but this is due to their being cut obliquely where they begin to bend down. The section is unfortunately rather badly preserved.

Section 18, fig. 34, Plate 3. Here the palatines are still more bent down. In this and the following three sections it is difficult to determine with certainty whether the slender ascending plates near the middle line belong to the palatines, the prevomers, or the pterygoids. In sections 15, 16, and 17 they seem to arise from the palatines. In the intermediate sections they appear to arise from the prevomers, but as in each section they are fractured it is impossible to be quite certain. Most probably the slender plates in this section, and in those following, are, as I have indicated in the figures, plates passing forward from the pterygoids above the prevomers.

Section 19, fig. 35, Plate 3, is better preserved than either of the two in front of it. We are here near the posterior end of the prevomers, which are seen as little plates on the palatal roof. The ascending median plates are probably pterygoid. One little detached plate on the left side is probably a sclerotic plate cut across. The palatines form most of the roof of the palate, and each has an ascending process which articulates with an inwardly passing process from the wall of the snout. It is, unfortunately, impossible to say with certainty what bone forms this lateral process, as most of the surface bones are completely weathered away, and no sutures can be made out. It seems most probable that it is a development from the anterior end of the jugal. The flat plates at the outer sides of the lower part of the section are the posterior portions of the maxillae.

Section 21, fig. 36, Plate 3, is through the extreme posterior end of the prevomers. Of the slender bones seen near the middle line, only two small parts appear to be prevomers. All the other slender elements are the anterior parts of the pterygoids. The palatines still form most of the palatal roof. The bony ridge seen passing in front of the outer left wall is probably part of the jugal. On the right side it is also probably jugal. Only sections of a more perfect skull will settle the matter.

Section 23, fig. 37, Plate 3. This section is fairly similar to the last one. The palatines here have no longer ascending plates. On the left side the palatine articulates with the maxilla. On the right side there appears to be a little element between the palatine and the maxilla. When the adjoining sections are examined, however, this is seen to be a portion of the palatine. From each outer wall an inwardly directed ridge is seen cut in section. These are probably formed by the jugals.

Section 25, fig. 38, Plate 3. Here, near the middle line, are seen the pterygoids with, on the left side, what is evidently a foramen. On the left side the palatine

articulates with the maxilla as in previous sections. On the right side the palatine is in two parts owing to the section passing through the anterior part of the suborbital vacuity. On the outer wall is seen a bony mass which is evidently mainly lacrimal above and in part jugal below.

Section 27, fig. 39, Plate 3. Here we meet for the first time the transpalatines. On the right side are seen just outside the suborbital vacuity three little bony elements. The middle one of the three is the transpalatine. The other two are parts of the palatine. On the left side the transpalatine is a well-developed element. The section is near the posterior end of the palatines. They are here relatively small, and the pterygoids are becoming larger. On the left outer wall are seen the prefrontal, the lacrimal, and the jugal, but the preservation is unsatisfactory.

Section 29, fig. 40, Plate 3. This section is through the front of the orbits. Above we have parts of the prefrontals and the frontals preserved. In the palate we have the pterygoids forming the middle portion, and the transpalatines seen at the outer sides. The lower portions of the orbits are evidently formed by the lacrimals. Above the pterygoid on the right side is seen a triradiating slender element. This can be seen in the following five sections. It is clearly the displaced and inverted and rotated pre-sphenoid, and this is the first time it has been observed in a Therocephalian skull.

Before proceeding further it seems advisable to say something about this little bone. In the Anomodonts (*Dicynodon*, etc.) we have a well-developed cartilage bone which lies below the frontal and lodges the olfactory portion of the brain. It has sometimes been called the ethmoid; sometimes the sphenethmoid. It is clearly homologous with a large bone similarly situated in the Pareiasaurians, which again is undoubtedly homologous with the bone called sphenethmoid in the frog. In the platypus we have a bone similarly situated in front of the brain, and though this has been called the mesethmoid, I have shown that there is no mesethmoid in the lower mammals, and that this bone is undoubtedly the pre-sphenoid. As stated above, it is well developed in the Anomodonts. It is unknown in the Dinocephalians but probably occurs. It is rather feebly developed in the Gorgonopsians. Among the Cynodonts it is unknown except in *Thrinaxodon*, where it is imperfectly ossified. Most probably it will be found well ossified in some of the larger types. That it should occur as a well-ossified though feebly developed element in the Therocephalians is very interesting. In the skull of the *Lycedops scholtzi* described later in this paper, it is seen in position.

Section 31, fig. 41, Plate 3. In this section the pre-sphenoid has the lateral wings more fully developed. The lateral parts of the section have been omitted to avoid overcrowding the plate.

Section 33, fig. 42, Plate 3. This section shows the pterygoids becoming thicker as they near the place where teeth are borne. The pre-sphenoid has here lost the median supporting base. Between the tips is a slender bone which is probably a sclerotic plate. On the right side the transpalatine is seen greatly enlarged, and stretching down to the place where it supports the pterygoid.

Section 34, fig. 43, Plate 3. This section is very similar to the last but shows a tooth in each pterygoid.

Section 35, fig. 44, Plate 3. Here the pterygoids are widening out as they approach the pterygoid process.

Section 36, fig. 45, Plate 3. This is the last section which shows the pre-sphenoid. As these last four sections show only the lateral wings of the pre-sphenoid, we are probably justified in concluding that the little bone is not only displaced and inverted but that the front of the bone is turned to the back. On the left side the pterygoid obscures the transpalatine, but on the right side the transpalatine is still seen.

Sections 37, 38, and 39, figs. 46, 47, 48, Plate 3. These sections are through the pterygoid-processes. Between the pterygoids in the middle line there is a hollow space partly filled by bony particles, but as this part is badly weathered it is impossible to say whether these bony fragments are parts of the pterygoids.

The sections behind Section 39 are all rather unsatisfactory in that the base of the skull is almost completely weathered away. Fortunately this region is much better preserved in the next series of sections to be considered. I figure three sections to show how closely the animals agree.

It will be observed that the posterior end of the transpalatine lies entirely in front of the outer process of the pterygoid. And this is the condition in every Therocephalian skull which I have examined where the bones are satisfactorily preserved. It is certainly the condition *Scylacosaurus*, *Trochosaurus*, *Lycedops*, *Hyenosaurus*, *Moschorhinus*, *Enchambersia*, *Ictidosuchoides*, *Hofmeyria*, and *Scaloposaurus*; and the pterygoid forms the back of the process in all known Gorgonopsians, Bauriamorphs, Dinocephalians, and Cynodonts. It is also the condition in lizards, *Sphenodon*, Pelycosaurs, and typical crocodiles. BOONSTRA (1935), in his recent paper on Therocephalians, while recognizing that it is the condition in *Scylacosaurus*, *Scymnosaurus*, *Trochosaurus*, *Ictidosuchoides*, and *Moschorhinus*, believes that in *Theriognathus*, *Notosollasia*, and *Whaitsia* the transpalatine (*ectopterygoid*) is so largely developed that it completely excludes the pterygoid from coming near to the mandible. If this observation is correct, it will mean that the higher Therocephalians are a little further removed from the early types than is generally believed.

I recently wrote to Dr. SWINTON to look into the point, and he replied as follows :—
“*Notosollasia* (R. 5699) is quite definitely and clearly as figured by BOONSTRA; I am of the opinion that *Theriognathus* (47065) is as he has it too. In *Whaitsia* it is a little different because the direction of the Ecpt-Pt suture, is as you have drawn it, but it seems to me to go to the posterior margin all right, and that the postero-lateral direction is due to the crushing the skull has suffered”.

When a few years ago I carefully considered the relations of the two bones in both *Notosollasia* and *Whaitsia* I came to the conclusion that in the latter the pterygoid forms the back of the outer part of the process. In *Notosollasia* the evidence was less clear, but as the two forms are closely allied, I saw no reason for doubting that in this respect the two are likely to agree. Whatever be the case with *Notosollasia*, *Theriognathus*, and *Whaitsia*, quite certainly the pterygoid forms the back part of the process in all the other Therocephalians whose palates are satisfactorily known.

Section 41, fig. 49, Plate 3. Here we see the pterygoids united and giving on section an x-like appearance. On the upper part of each pterygoid is a small element which is the anterior end of the base of the epipterygoid. In the upper part of the section we see the frontals, post-frontals, and post-orbitals cut across.

Section 45, fig. 50, Plate 3. The upper part of this section is through the pineal foramen. The lower part shows the base of the epipterygoid a little distance in front of the ascending plate. It rests on the pterygoid and on part of the lateral wing of the vomer (parasphenoid).

Section 47, fig. 51, Plate 3. This section of the pterygoid complex is about 7 mm behind section 45 and immediately in front of the ascending plate. The arrangement of the bones is similar to that in the preceding section. It is not quite clear how far down on the side of the vomerine wing the pterygoid passes.

In sections 48, 49, and 50 we have the ascending plate of the epipterygoid. Owing to much weathering and imperfection these posterior sections are unsatisfactory.

V—TRANSVERSE SECTIONS OF THE BACK PART OF SKULL OF A THEROCEPHALIAN— PROBABLY *Trochosuchus acutus*, BROOM

This specimen, which was cut into a series of slabs, is the imperfect and considerably weathered posterior third of a moderately large Therocephalian. As no part of the anterior two-thirds was preserved, the species cannot be determined with certainty. The specimen was picked up in the same locality and horizon as has yielded a specimen of *Trochosuchus acutus*, and there seems reason to believe that it belongs to this species. Certainly if not identical, it is a closely allied species.

As preserved, the specimen did not seem at all promising. One-half of the base of the skull was weathered away, with the whole of the squamosal region of the left side. Not thinking the specimen was likely to prove of much value, I had it cut into rather thick slabs, each of which is about 8 mm in thickness, and each cut has removed about 5½ mm of the specimen. Fortunately, the sections showed that the interior of the specimen is in excellent preservation, and the sections are thus of great importance.

Section 1, fig. 52, Plate 4. The first section that seems worth figuring is immediately behind the orbit and along much of the post-orbital arch. The frontal bones are here rather narrow, and outside the frontal is seen the very large post-frontal which forms the greater part of the upper third of the post-orbital arch. Outside of the post-frontal and partly below it is seen the relatively small post-orbital. And clasped by the post-orbital on its outer side is the upper end of the jugal. In the lower part of the section, which unfortunately is imperfect, especially on the left side, are seen, in the middle line a section of the anterior part of the vomer (para-sphenoid), and on the right side a little bony complex of which the outer and lower part is pterygoid, and the upper part the anterior end of the basal portion of the epipterygoid, and internal to the epipterygoid is a small portion of the wing of the vomer (para-sphenoid).

Section 2, fig. 53, Plate 4. This section, which is about 8 mm behind section 1, is completely behind the orbit on the right side. Owing to all the sections having been cut a little obliquely, it would cut into the post-orbital arch on the left side were this completely preserved in the specimen. The section cuts through the suture between the frontals and the parietals. Most of the bones near the middle line are parts of the frontals. Outside the lower parts of the frontals are seen portions of each parietal. On the left side is a part of the post-frontal; and on the right side a part of the post-orbital and of the post-frontal. In the lower part of the section are seen two parts of the vomer (para-sphenoid), a large part of the base of the epipterygoid, and a considerable part of the pterygoid.

Section 3, fig. 54, Plate 4. This section, which is about $5\frac{1}{2}$ mm behind the last, is very similar to it. The main bones of the upper cranial wall are the parietals, only a few small parts of the frontals being seen. On the right side are small parts of the post-orbital and of the post-frontal. On the left side are seen a fair-sized part of the frontal, and parts of the post-frontal and post-orbital. In the lower part the vomer is seen as a median grooved portion, and with lateral wings which support the pterygoids and epipterygoids. The basal portion of the epipterygoid is well developed and rests on the pterygoid and vomer. The pterygoid is a slender curved bone which passes down on the outer side of the vomerine wing.

Section 4, fig. 55, Plate 4. This section is about 8 mm behind section 3. The upper part of the section is chiefly made up of the large parietals fused together. On each side are parts of the post-frontals and post-orbitals, and on the left side is a very small part of the left frontal still seen. Below, we have near the middle line two parts of the vomer (para-sphenoid); the part on the left side supporting the epipterygoid, though the suture is not clearly preserved. On the right side both the pterygoid and the epipterygoid are free from the vomer, but they are still articulated to each other. The upper part of the epipterygoid is fractured, but I have drawn it as it is seen in the specimen. Farther out on the right side is a section of the mandible near the articulation. The central element is the large articular. On its inner side is seen a section of the pre-articular, and on its outer side a section of a considerable portion of the sur-angular. Below is a small section of the angular.

Section 5, fig. 56, Plate 4. This section, about $5\frac{1}{2}$ mm behind section 4, is fairly similar to it. The upper part of the section is almost entirely composed of the parietals with, on the left side, small parts of the post-frontal and of the post-orbital. In the middle line, below, we have part of the vomer (para-sphenoid), and on the left side part of the base of the epipterygoid. On the right side is seen part of the ascending plate of the epipterygoid fractured near the middle. The lower part is seen articulating with the slender pterygoid. Between the upper part of the epipterygoid and the parietal is seen a small bony element, the greater part of which is seen in the next two sections. This element is, I believe, the pleuro-or latero-sphenoid. Outside the pterygoid is another section of the mandible. The main element is the articular. On its inner side it is clasped by the pre-articular; and on its outer side is seen a portion of the sur-angular.

Section 6, fig. 57, Plate 4. This section is about 8 mm behind section 5. The upper part of the section shows the two parietals with a large space between for the upper part of the pineal organ. Below the parietals there are seen passing out on each side portions of the ascending plates of the epipterygoids. On the left side the lower part of the epipterygoid is fractured, and there is a very distinct oval foramen in the upper part, possibly for a vein. On the right side there is a little part of the lower end of the epipterygoid seen in close association with the pterygoid. Between the epipterygoids are four portions of the pleuro-sphenoids. Inferiorly, in the middle line, we have a part of the basi-sphenoid. On the right side there is an elongated element lying between the basi-sphenoid and the articular. This is probably the slightly displaced stapes. The articular is broad and flat. Above it is a small portion of the quadrate.

Section 7, fig. 58, Plate 4. This section, which is about $5\frac{1}{2}$ mm behind section 6, is of great interest. The parietals are here narrow and anchylosed together. On their upper end is a small hollow, which is part of the pineal foramen. On the right side only a part of the upper end of the ascending plate of the epipterygoid is seen. On the left side there are three parts of the epipterygoid seen. Nearer the middle line are seen the main parts of the pleuro-sphenoids. The bones are very delicate and spongy. On the inner side of each bone is a foramen most probably for the sixth nerve. Each bone rests on the basi-sphenoid. Apparently these bones have formed the supports to a large hypophysis, and apparently they have, to a considerable extent, supported the brain above. On the right side of the basi-sphenoid is a small bony element which is apparently part of the prootic. A little farther out is a fragment of what is evidently the displaced stapes. Still farther out is the large quadrate. In close contact with it below is the flat posterior end of the articular. On the inner side of the quadrate is a section of the posterior end of the pterygoid.

Section 8, fig. 59, Plate 4. This section, which is 8 mm behind the last, is cut through the anterior part of the opisthotic. Below the parietal are two portions of the anterior upper ends of the supra-occipital. In the middle line, below, we have a section of the basi-occipital with, above it, portions of the two prootics. The large bony mass on the right side is probably all opisthotic, and the space between it and the basi-occipital and prootic is the anterior part of the labyrinth cavity; but the details of the outer wall cannot be satisfactorily made out. Above the outer part of the opisthotic is a small portion of the squamosal, and outside the squamosal a large part of the quadrate. Outside the quadrate is a minute bony flake which is apparently part of the quadratojugal.

Section 9, fig. 60, Plate 4. This section, which is only about $5\frac{1}{2}$ mm behind the last, is very similar to it, and most of the bony mass on the right side is apparently the opisthotic. But the upper inner part is probably prootic. Above its outer end is a section of the squamosal, and outside the squamosal a section of the upper end of the quadrate. A small bit of the squamosal is seen below the quadrate. Near the middle line is a part of the basi-occipital, and between it and the opisthotic is another section of the labyrinthine cavity. A portion of the prootic is seen on the left side.

Between this prootic of the left side and the basi-occipital the base of the skull is badly weathered and part lost.

Section 10, fig. 61, Plate 4. This section, about 8 mm behind the last, cuts the brain cavity in the region of the jugular foramen. In the upper part of the section we have the conjoined parietals here broadening out and resting on the well-developed supra-occipital. Farther down we have on the left side a part of the prootic, and on the right side a part of the lateral portion of the supra-occipital. Below the supra-occipital, we have the well-developed par-occipital process of the opisthotic. Articulating both with the supra-occipital and the opisthotic is a section of the squamosal. The space between the squamosal, the supra-occipital, and the opisthotic is the entrance to the lateral occipital foramen. In the lower part of the section is a part of the basi-occipital, and resting on it a very small part of the exoccipital. Between the occipital and the opisthotic is the foramen for the ninth, tenth, and eleventh nerves. The hollow on the inner side of the left is probably for the lodgement of a flocculus.

Section 11, fig. 62, Plate 4. This section is very similar to the last. In the upper part we have the interparietal appearing between the parietal and the supra-occipital. On the left side the supra-occipital still articulates with apparently the prootic. On the right side the supra-occipital meets the squamosal and the opisthotic; while the exoccipital articulates with the opisthotic. In the outer part of the section we see the squamosal supporting the par-occipital process.

Section 12, fig. 63, Plate 4. This section, 8 mm behind the last, is through the foramen magnum. Above we have the parietals still widening out and resting on a large interparietal. The interparietal completely overlaps the upper part of the supra-occipital, and this section shows part of the supra-occipital, in the interparietal. The lower part of the section is mainly formed by the supra-occipital. On the left side is seen a small part of the opisthotic, and on the right side a small part of the exoccipital. On the outer part of the right side is seen a section of the squamosal.

Section 13, fig. 64, Plate 4. This section is through the occiput quite behind the brain cavity. Above, we see the parietals supported by the median interparietal. Below this we see on each side sections of the lateral plates of the supra-occipital. On the right side we still have a section of the squamosal and a small part of the tabular. On the left side below the supra-occipital we have what appears to be a small portion of the exoccipital.

VI—HORIZONTAL SECTIONS OF THE POSTERIOR PART OF THE SKULL OF A THEROCEPHALIAN—PROBABLY *Lycedops scholtzi*, BROOM

The specimen from which these sections were cut was a skull which had lost the front half of the snout, and with the remaining part of the snout badly weathered. As it seemed hardly worth while having the snout sectioned, I arranged to have the posterior part of the skull cut as this, though embedded in a very hard matrix, appeared to be well preserved. Owing to a misunderstanding, the specimen was cut

horizontally instead of transversely, as I had wished. The mistake, however, has proved a very fortunate one, and has resulted in a clearer understanding of the structure of the posterior part of the skull than would have been possible by transverse sections. The horizontal sections are not only in the plane of the frontal and parietal but in the plane of the base of the skull.

Section 1, fig. 65, Plate 5. This section is through the top of the parietal intertemporal crest, and through the top of the post-orbital arch of the left side. It is interesting as showing that the interparietal does not appear on the top of the crest. The pineal foramen is seen to be well developed. The three bones of the post-orbital arch seen are the post-frontal, forming much of the orbital margin; the post-orbital behind it; and a small part of the jugal.

Section 2, fig. 66, Plate 5. The parietal is very similar to that in the previous section, and there is still no evidence of the interparietal. Parts of each frontal are seen in section. On the left side an oval vacuity is seen. This is due to the surface of the bone being concave. Almost the whole extent of the left post-frontal is seen in the section, and behind it is a large part of the post-orbital and a small part of the jugal. On the right side are seen two parts of the post-frontal and the narrow upper edge of the post-orbital.

Section 3, fig. 67, Plate 5. This section shows the upper edge of the interparietal lying behind the parietal except in the middle line. On the outer side of the left parietal at the posterior end is seen the upper and inner end of the squamosal. Much of the post-orbital arch of the left side is seen. The post-orbital is in three parts; the division between the inner two parts is doubtless due to a crack in crushing. A part of the upper end of the jugal is also seen. The post-frontal here still forms much of the orbital wall. Both frontals are well seen, and a vacuity seen in the right one owing to the concavity of its surface. The right post-orbital bar is seen mainly formed by the large post-frontal.

Section 4, fig. 68, Plate 5. In this section the parietal is larger than in the upper sections. In front of the pineal foramen the two parietals are much expended, and there is a marked suture between them, though behind the foramen they are completely fused. On the occipital end of the parietal is seen the large interparietal, and farther out, on the left side the tabular; but in this section the suture between the tabular and interparietal cannot be made out with certainty. On the front of the outer end of the occipital plate of the parietal is seen the inner plate of the squamosal. In front of the parietals are seen parts of the posterior ends of the frontals. Inner and outer portions of the left post-orbital arch are seen. The inner end is only a small part of the post-frontal; and the outer, parts of the post-orbital and jugal. On the right a large part of the post-frontal is seen, and a very small part of the post-orbital.

Section 5, fig. 69, Plate 5. This section is very similar to the previous one, but the pineal foramen is no longer surrounded by bone. On the left side a large part of the tabular is seen, and a smaller part of the squamosal. The interparietal is well shown.

Section 6, fig. 70, Plate 5. Here we are approaching the lower part of the parietal, though it is still of large size. Behind it in the middle line is the well-developed inter-

parietal excavated from below, where it approaches the supra-occipital. On the left side the large tabular is seen connecting the squamosal with the parietal and interparietal. In front no part of the left post-orbital arch is seen ; but on the right side we see a large part of the post-orbital and small portions of the post-frontal and jugal.

Section 7, fig. 71, Plate 5. This section is through the base of the parietal, of which considerable portions are still seen. Behind it the interparietal is seen narrower than in the earlier sections. On each side the tabulars are seen as large elements and articulating with the squamosals. In front of the interparietal on the left side is a section through the top of the supra-occipital. Parts of the jugal and post-orbital represent the right post-orbital arch.

Section 8, fig. 72, Plate 5. This section is almost entirely below the parietal, only a tiny portion of the bone being seen on the right side. The top of the supra-occipital is seen as a horseshoe-shaped bone. The small interparietal is closely articulated to it behind. On both sides large parts of the tabulars are seen. In front of the supra-occipital on the left side we have a section of the top of the epipterygoid. A small part of the right post-orbital arch is seen.

Section 9, fig. 73, Plate 5. This is essentially similar to the previous section. Here sections of each epipterygoid are seen.

Section 10, fig. 74, Plate 5. This section is interesting from being mainly through the suture between the interparietal and the supra-occipital. In the middle line a small part of the interparietal is still seen resting on the supra-occipital. On the left side we have the tabular being excavated below by the supra-occipital. On each side the squamosals are seen articulating with the tabulars.

Section 11, fig. 75, Plate 5. In this section the interparietal is no longer seen, and the tabulars are smaller than in earlier sections. On the left side much of the occiput is formed by the supra-occipital. The tabular is partly wedged in between the supra-occipital and the squamosal. In front of the supra-occipital the epipterygoids are seen in section.

Section 12, fig. 76, Plate 5. Here on the left side only the lower end of the tabular is seen resting on the squamosal. On the right side the tabular is fairly large, but has the supra-occipital seen through the middle portion as on the left side of the section 10. In front are the two epipterygoids. Between that of the left side and the supra-occipital is the top of the petrotic mass which may be regarded as probably prootic.

Section 13, fig. 77, Plate 5. Here the whole of the middle part of the occiput is formed by the supra-occipital. On the left side the tabular is no longer seen, but on the right we still have a considerable part. In front of the supra-occipital on each side are seen the upper parts of what are probably prootics. A little further in front we have sections of the epipterygoids. Inside each epipterygoid is a little bony element which is continued down into the prootic. It is the rudimentary pleuro- or laterosphenoid.

Section 14, fig. 78, Plate 5. This section is very similar to the preceding one.

Section 15, fig. 79, Plate 5. Here on the occipital face are seen on the right side the squamosal, tabular, and supra-occipital ; and on the left side are seen the

supra-occipital, periotic mass, and squamosal. It is impossible to trace a suture between the prootic and opisthotic. In front of the prootic are two small elements, the right attached to the prootic. These are the bases of the pleuro-sphenoids. Sections of the epipterygoids also are seen.

Section 16, fig. 80, Plate 5. This section and the next six sections are shown in Plate 5 of larger size, owing to their great importance. I have indicated all the sutures that are manifest, but those between the prootic and opisthotic are not quite clear. In this section the tops of the exoccipitals are seen, with behind them still considerable portions of the supra-occipitals. On the right side the outer limits of the supra-occipital are uncertain. There is a clear suture as indicated between the supra-occipital and what is probably prootic, but articulating with the squamosal farther out is a small portion of what is probably also supra-occipital; but no suture can be traced between this and the outer part of the prootic.

On both sides we have sections of the top of the cavity for the labyrinth. On the right side the walls of the cavity appear to be formed by the prootic in front and the opisthotic and supra-occipital behind.

In the anterior parts of the prootics are still seen the bases of the pleuro-sphenoids. A little farther back on the left side is seen a fairly large, round opening. This is evidently the cavity in which lay the saccus endolymphaticus. In the following section, fig. 81, the cavity is seen united with the general cavity for the labyrinth. Sections of the epipterygoids are seen near their bases.

Section 17, fig. 81, Plate 5, and fig. 88, Plate 6. This section is about 3 mm below section 16. Posteriorly are seen sections of the exoccipitals on the two sides of the foramen magnum. Behind each is seen what appears to be a portion of the supra-occipital, and behind and outside these lower portions of the supra-occipital are sections across the upper parts of the opisthotics. The outer of the two concavities on the left side about the middle of the prootic is that for the saccus endolymphaticus. Though the labyrinth cavity is widely open into the brain cavity, there are indications of an imperfect wall between the cavities, but the detailed structure could only be made out by having a continuous series of close sections by Sollas's method, which would mean the complete loss of the slabs for any other study. Two large sections of the bases of the epipterygoids are seen. And well in front is a section of the anterior process of the vomer (para-sphenoid).

Section 18, fig. 89, Plate 6. This horizontal section is through the lower part of the brain cavity, and the main part of the labyrinth, and about the middle of the foramen magnum. Posteriorly the two bones bounding the foramen magnum are the exoccipitals. In front of the exoccipital on the right side is the large opisthotic, and in front of this is the prootic. On the left side the opisthotic is apparently very much smaller, but there is some doubt as to the position of the suture between the prootic and the opisthotic. The dotted line on the figure indicates what is probably the division between the bones.

On each side are seen the irregular spaces for the labyrinths, but I am unable to make out much of the structure of the labyrinth from the few bony processes and

hollows. Had the sections been more closely cut doubtless we should have been able to make a fair restoration of the inner ear. In front of the exoccipital on the left side and behind a process from the opisthotic is a large oval space. This is part of the jugular foramen for the ninth, tenth, and eleventh nerves.

The prootics approach each other in the middle line, and between them in front is a small part of the basi-sphenoid. In the front part of the section are seen portions of the vomer and of the pterygoids ; and laterally we have sections of the epipterygoids.

Section 19, fig. 90, Plate 6. This section is through the upper part of the floor of the brain-case. In the middle line we have sections of the basi-occipital and basi-sphenoid. On either side of these bones are seen the prootics, with behind the prootics the large opisthotics. The right side of the section agrees fairly closely with the left side of the preceding one. We have the cavity for the labyrinth in front, and the oval foramen for the ninth, tenth, and eleventh nerves. On the right side we have the foramen for the hypoglossal nerve passing through the exoccipital. In front we have a large section of the vomer, with in front of it sections of the pterygoids and outside of these sections of the epipterygoids.

Section 20, fig. 82, Plate 5, and fig. 91, Plate 6. This section is through the middle of the floor of the brain-case. The basi-occipital forms most of the floor. In front of it is seen the basi-sphenoid with the large vomer ankylosed to it. Though there is no suture between the bones, the two bones are very distinct as the vomer is dense bone and the basi-sphenoid spongy bone. Laterally we have the exoccipitals, the opisthotics, and the prootics. In front the vomer articulates with the pterygoids, and on the left side is still seen part of the epipterygoid. The whole section is very mammal-like.

Section 21, fig. 83, Plate 5, and fig. 92, Plate 6. This section is through the lower part of the floor of the brain-case. On the whole, the section agrees with the preceding one. The vomer is large, and is seen articulating laterally with the basi-occipital. A small part of the basi-sphenoid is seen near the hind part of the vomer. The cavity for the vestibule is still seen on each side.

Section 22, fig. 84, Plate 5, and fig. 93, Plate 6. This section is just below the floor of the brain case, and through the large columnar processes at the end of which are the fenestrae ovals. The vomer is seen in four sections. The anterior median one is through the vertical pharyngeal plate ; the two lateral portions are supporting plates against the fronts of the columnar processes ; and the tiny median portion is the posterior end of the vomer which lies against the basi-occipital near the condyle.

The fenestrae ovals are seen nearly surrounded by processes from the basi-occipital and opisthotics.

In fig. 84 we have sections across the back part of the mandible and through the quadrate. The mandibular section shows sections of the articular, pre-articular, and angular. The quadrate is in two portions, but this is doubtless due to a fracture.

Section 23, fig. 85, Plate 5. This section resembles the preceding one so closely that it is unnecessary to comment on it.

Section 24, fig. 86, Plate 5. In this section the only portions of the median elements are a section of the median plate of the vomer, and a tiny section of the pterygoid. The section is through the hinge of the jaw, and shows the articular, pre-articular, quadrate, and probably a portion of the small quadratojugal.

VII—THE SKULL OF *Lycedops scholtzi*, BROOM

In the McGregor Museum, Kimberley, there is a skull of a Therocephalian from the *Tapinocephalus* zone, which I have just made the type of a new genus and species. Though the skull is very considerably weathered, and a portion of the snout in the canine region is missing, it shows satisfactorily a number of details of the Therocephalian structure which were less satisfactorily or not at all shown in the sections that have just been described.

In the transverse sections of the skull of *Pristerognathus minor*, the very feebly developed pre-sphenoid was figured in a considerable number of sections, but displaced from its natural position. In a natural fracture across the skull of *Lycedops* we have the pre-sphenoid in its undisturbed relations with the frontals, fig. 96, Plate 7. It is seen as a ring of bone, or rather as two arcs, which meet below. This section is doubtless through the anterior part of the bone where there is no basal support.

In the lower part of the section, which is slightly oblique, the right-hand side of the figure being very appreciably farther forward than the left, are seen sections through the wide transverse portions of the pterygoids. On the right the pterygoid articulates with the transpalatine, but in the section, the part where the two bones meet has been weathered off. The transpalatine is a deep, rather powerfully developed bone. Above, the transpalatine articulates with three small elements. The larger of these just outside the upper end of the bone is manifestly the jugal, and I think the two small bony structures round the top of the little median element are also parts of the jugal. The small median element is probably a posterior process of the palatine.

A very interesting section of the mandible is seen on the right side. The dentary is cut just a little in front of the angular process, with the upper part, which is the base of the coronoid process, powerfully developed, and the lower part rather feebly. Inside the dentary are seen five elements in section. The two near the lower part of the dentary are the anterior part of the sur-angular above, and a section near the anterior end of the angular below. The three bones lying one above the other more internal, are the coronoid above, the splenial below, and the pre-articular in the middle. On the left side of the section is seen a cut across the mandible considerably farther back. Here the dentary is seen above, with below it the sur-angular and the angular. Internal to the angular are sections of the pre-articular, coronoid, and the splenial.

Two other sections of the mandible are shown. In fig. 97 we have a section fairly similar to that seen on the left side of fig. 96, but cut in a slightly different plane.

Here we have still the same six elements. The coronoid is fairly large and the sur-angular small. In fig. 98 we have a section appreciably farther back. Here the sur-angular is powerfully developed and the angular much the largest bone of the section. The splenial is cut near its posterior end and appears as a very tiny element. The pre-articular is rather better developed than in the other sections shown, and the coronoid is cut near its posterior end.

Fig. 99 shows a somewhat oblique and irregular section through the base of the skull. Above, we have the parietals a short distance behind the pineal foramen. Below it are seen sections of the epipterygoids. In the lower part of the figure we have portions of the basi-occipital, the opisthotics, and the prootics cut across and on each side we have the vestibules in section. There is seen on each side an upward development from the prootic. This is the pleuro- or latero-sphenoid which will be more fully discussed later. There is clearly seen a foramen on one side. This is probably the foramen for the sixth nerve.

Fig. 100, Plate 7, shows a side view of the vomer (para-sphenoid) with its articulations with the pterygoid in front, the epipterygoid above, and the basi-occipital and basi-sphenoid behind. As in the sections figured, the small basi-sphenoid is a porous bone, but completely anchylosed with the denser bone of the vomer. The anterior rostral process of the vomer is not displayed in the section of which only the lower part is median. Most of the pterygoid and the lateral process of the vomer are considerably to the side.

In fig. 102, Plate 7, we have the occiput very satisfactorily shown. Above, in the middle line, we have the fairly large interparietal, and laterally to it we have the tabulars. Below the interparietal we have the large, wide supra-occipital. This forms the upper part of the foramen magnum, and extends laterally to the squamosals. Below the outer part of the tabular the occiput is deeply depressed. In the lower part of the depression and between the supra-occipital and the opisthotic is the moderately large oval opening through the occiput. The opisthotic is rather peculiarly developed. The outer end is very broad and curved. The posterior part has a large articulation with the squamosal. The anterior gives an articulation to the quadrate, and between the two parts of the outer end is a deep depression. The tabular and the squamosal both overlap the outer part of the supra-occipital. It is a little difficult to trace the exact sutures as the squamosal and tabular in this region are reduced to very thin flakes of bone. The exoccipital is moderately developed. It has a long articulation with the supra-occipital and the opisthotic as shown in the figure.

The relations of the squamosal, quadrate and quadratojugal are for the first time in a Therocephalian fully revealed in this skull. The squamosal is a somewhat fan-shaped structure. The sections already described reveal the relations of its inner part to the parietal and tabular. Here we see the structure of the lower part. A ridge runs down from the post-temporal margin of the occiput, and forms the outer wall of the lateral occipital depression. On the outer side of this ridge is a groove in which doubtless lay the external auditory canal, and this becomes more certain as we can

see clearly how the tympanic membrane has been attached. Below the squamosal we have the quadrate which is rather curiously shaped. Its articular end is very broad and roller shaped. Its inner side is sharp and curved, and there can, I think, be no doubt that the tympanic membrane was attached to this curved edge of the quadrate. Above, we have the rounded edge of the lower border of the squamosal, and this edge with the curved edge of the quadrate, which is practically continuous with it, forms about three-quarters of a circle. Further, the stapes are preserved in position on both sides of the specimen. The head differs from that of all hitherto known Therapsid stapes in having, in addition to a large articulation with the quadrate, a well-marked process which doubtless gave articulation to a cartilaginous extrastapedial which most probably was attached to the tympanic membrane.

The quadratojugal is perfectly preserved on one side. Fig. 103, Plate 7, shows the posterior aspect of the bone. It has a wide articulation with the lower part of the quadrate, and an upper inner process meets the upper outer process of the quadrate, and between them we have a typical quadratojugal-quadrates foramen. Immediately outside the tympanic region of the squamosal are two descending short processes. Of these the outer one rests on the quadratojugal. A section across this region is shown in fig. 104, Plate 7. Here we see how the upper part of the quadratojugal lies between the quadrate and the squamosal, and how the upper part of the quadrate closely clasps parts of both bones. In this same figure is seen an oblique section of the articular end of the mandible.

Fig. 95 shows the side-view of the skull, with the outer side of the mandible in almost perfect preservation. The dentary forms about two-thirds of the jaw, and is remarkable for the rather thick, abruptly truncated coronoid process. The angular is very large and, as in most Therapsids, the bone consists of an inner main plate and a thin outer fan-like plate. This outer plate is peculiarly folded; and very manifestly the outer plate has been for the protection of some organ that lay between the two plates. Some years ago I suggested that this may have been a submaxillary gland, and I still think this is the most likely explanation. The underside of the cavity is always freely open, while the top of the cavity is only partly open as if for blood vessels.

Above the angular is seen part of the rather stout, curved sur-angular, and behind it is seen part of the articular. But though the articular appears small from the outside, it is broad and has a large transversely hollow articulation for the quadrate.

Fig. 101, Plate 7, shows the probable appearance of the inner side of the jaw restored from sections of the allied *Pristerognathus minor*, and from the sections of the jaw of *Lycedops*, and from the parts seen in the specimen. The only points that remain in doubt are the exact shape of the coronoid, and how far forward it and the pre-articular extend. The most striking characteristic of the jaw is the great extension backwards of the splenial. In this the early Therocephalian differs markedly from the Gorgonopsian, but in the main agrees with the Cynodont, and to some extent with the Dinocephalian. The coronoid is essentially similar to that of the Cynodonts and Gorgonopsians.

The mandible in having a well-developed coronoid process is less primitive than that of the Dinocephalian, but it is essentially of the same type. The Anomodont is so specialized in the structure of the articular and the absence of canines that it is difficult to determine what characters are primitive and what are secondary specializations. Most likely the ancestral Anomodont had a coronoid process and also a coronoid bone, and if we one day find the jaw of a pro-Anomodont we shall probably find that it resembles the jaw of the Gorgonopsian rather closely. The Therocephalian jaw differs in the greater development of the back of the dentary, and the less development of the anterior parts of the angular.

The resemblances to the jaw of the Cynodont are very interesting, and suggest the possibility of the Cynodont perhaps having been evolved from a Therocephalian rather than from a Gorgonopsian, as we have hitherto suspected. The great posterior development of the splenial and the very short anterior development of the angular are characters common to the Therocephalians and the Cynodonts.

The pro-atlas, atlas, and much of the axis are preserved, though they have not been completely cleared of matrix. The pro-atlas is a small plate. The atlas is very similar to that in the Tapinocephalids, the Anomodonts, and the Gorgonopsians in that the two halves of the arch are quite free from each other, and only loosely attached to the basal portion presumably by ligament. In the Titanosuchids the two sides of the arch are fused, and they are closely attached to the basal portion. In the Cynodonts the halves of the arch are also united, but they are not closely articulated with the basal element.

The axis has the odontoid closely articulating with its centrum, but not at all anchylosed. A small intercentrum lies below the articulation of the odontoid with the centrum. There are probably small ribs on both atlas and axis.

VIII.—THE SKULL OF *Hofmeyria atavus*, BROOM

This little skull, recently discovered by me near Biesjespoort in beds which are either at the top of the *Endothiodon* zone, or the base of the *Cistecephalus* zone, is of very great interest as no very good Therocephalian skulls have hitherto been found at any horizon from the base of the *Endothiodon* zone to about the middle of the *Cistecephalus* zone. A small Therocephalian *Ictidognathus parvidens*, Broom, is known from the base of the *Cistecephalus* zone, but is not sufficiently well preserved to reveal much of the structure, and another skull *Ictidosuchoides longiceps*, Broom, is known from beds that are also probably near the base of the *Cistecephalus* zone, but this lacks the mandible, and the premaxillae and nothing is known of the occiput.

From the base of the *Endothiodon* zone to the very top, only one fairly satisfactory Therocephalian skull is known, *Choerosaurus dejageri*, Haughton, and this is only known by its external characters, and those are not altogether satisfactorily preserved, and though the form is primitive, in a number of characters it is highly specialized. It may be related to the Scaloposaurids.

This newly discovered type resembles the early *Terocephalians* more than the later forms, but it is possibly a connecting link between the primitive types and the Upper *Cistecephalus* zone forms.

The skull is only about 3 inches in length, and fortunately is beautifully preserved, the only serious defect is that the right side of the occiput with the articular region is somewhat crushed forwards. Owing to the importance of the type, I have given various figures of the skull as preserved and restorations of the side, top, and occiput, with the distortion corrected. In perfect condition the skull probably measured in greatest length 78 mm and in greatest width 50 mm.

The premaxillary bones are small, and there are five incisors which measure 10 mm. The septomaxillary is very short, and the posterior process between the nasal and the maxilla shorter relatively than in any other known *Terocephalian*. The maxilla is relatively short but strongly built. It carries one small canine and five small, pointed molars. The nasal bones are short and broad, and fairly mammal-like. The pre-frontals are large, and each forms a well-marked tumescence in front of each orbit. The lacrimal is a little smaller than the pre-frontal, and there are, just within the orbit, two lacrimal foramina, as shown in fig. 114, Plate 8. The post-frontal bone is larger than the post-orbital, and each post-frontal forms a large part of the orbital margin, just meeting the pre-frontal and shutting out the frontal from the orbital margin. The post-orbital forms much of the post-orbital arch. The relations and shapes of these bones are shown in the figures.

The frontals are short and broad. Posteriorly they meet the parietals by a transverse serrated suture. The parietals are united and form a short, rather low parietal crest. There is a moderate-sized pineal foramen. Posteriorly, the parietals pass well outwards, forming much of the occipital crest, and in the middle region they form the upper part of the occiput, so that the interparietal does not appear on the upper surface of the skull.

The jugal is long and slender, and it forms much of the lower part of the post-orbital arch. The squamosal is moderately large. Its outer anterior part spreads over the jugal to form the back of the temporal arch. The inner part passes upwards and inwards, meeting and overlapping the parietal and forming a considerable part of the occipital crest. Posteriorly, the squamosal forms a considerable part of the occipital face. It articulates with the opisthotic, and just outside this articulation is the marked descending auditory groove. Above, the squamosal has a large articulation with the tabular, and apparently a small articulation with the outer part of the supra-occipital below the tabular. Inferiorly, the squamosal supports the quadrate and the quadratojugal, though, owing to crushing and the very small size of the bones, the details of structure cannot be so satisfactorily made out as in *Lycodops scholtzi*. But apparently the condition is very similar.

The interparietal is a small bone situated near the top of the occipital face, but entirely confined to this occipital surface. It lies between the parietal, the tabulars and the supra-occipital. It is almost three times as wide as deep. The tabular is a well-developed bone which forms the upper and outer portions of each side of the

occiput. The supra-occipital is a very broad, shallow bone. Above, it articulates with the tabulars and the interparietal and below with the exoccipitals and the opisthotics. The exact relations of the outer end cannot be determined with certainty. Probably it meets the squamosal underneath the tabular, and forms a small part of the margin of the large occipito-temporal foramen. The opisthotic forms a peculiar paroccipital process. This, at its outer end, is curved upwards to give a long articulation to the squamosal. Above this process is the large foramen, and the opisthotic certainly forms the lower, inner, and greater part of the upper walls of the foramen.

On each side of the foramen magnum is a moderately large exoccipital, which sends out a large process along the posterior side of the opisthotic. Between the inner side of the opisthotic and the exoccipital is a fairly large jugular foramen. Below the opisthotic is seen on each side a slender stapes, which extends from the fenestra ovalis to the inner end of the quadrate. We have seen in *Lycedops scholtzi* that the outer end of the stapes, in addition to articulating with the quadrate, has an upper development of bone which may be the base of some extra-stapedial structure. Here, in *Hofmeyria*, we again see, but much more clearly, what are manifestly the remains of some extra-stapedial structures. On the right side we find passing upwards from the distal plate which articulates with the quadrate, a little bony process which is about half as long as the whole mediostapedial. Another little bony spur passes inwards from the upper process. Of course, as these extra-stapedial structures are not so thick as a horse-hair it is impossible to trace them out fully. On the left side the conditions are a little different. Here the upward process from the outer end of the mediostapedial is directed mainly outwards. It seems, there can be little doubt, that in the Therocephalians there is a rod-like bony mediostapedial passing from the fenestra ovalis to the inner process of the quadrate, while from the upper side of the distal plate a delicate bony structure passes towards the tympanic membrane. Whether the whole of the extra- or supra-stapedial structure is bony is unknown. Very probably it was in part cartilaginous, as in the fowl or lizard. But clearly the tympanic membrane has been attached to the lower inner part of the squamosal, and along much of the inner part of the quadrate; and while the stapes articulates at its outer end with part of the quadrate an extra- or supra-stapedial structure, in part bony but possibly also in part cartilaginous, most probably was attached to the tympanic membrane.

While this important new light on the structure of the middle ear in the Therapsids does not completely solve the problem of the evolution of the mammalian middle ear it does bring us nearer to a definite solution.

In 1912 (Broom, 1912, p. 424), when dealing with the auditory region in *Dicynodon*, I gave a number of diagrams of the possible steps in the evolution of the mammalian auditory ossicles. The new evidence seems to be quite in harmony with the views then expressed. The position in which I placed the tympanic membrane proves to be correct; and the only important modification required in these diagrams is that an extra or supra-stapedial lay between the tympanic membrane and the top of the stapes while the new auditory ossicles were evolving.

The palate of *Hofmeyria atavus* cannot be completely cleared, as the mandibles are in position and make the development of the anterior part impossible, while in the back part we have the hyoid apparatus preserved. It has been possible, however, to clear the middle portion, and the rest can be restored with some probability.

The transverse processes of the pterygoids are well developed, but the anterior processes are rather slender. There is a median vacuity between the two pterygoids, and a little in front of the vacuity is a median boss which has on either side a deep depression. Outside each depression is a short ridge which carries a few teeth.

There is a relatively small suborbital vacuity with, on its outer side, a short, broad transpalatine. The palatines are evidently of large size, but only the posterior halves can be seen.

The lower jaws are well preserved ; that of the left side almost perfectly, but only the outer structure can be seen. The dentary is short and much curved, and though at first sight there appears to be a large coronoid process, the process only rises a very short distance above the sur-angular.

The angular is essentially similar to that of *Whaitsia major*, but differs very considerably from that in *Lycedops* and the other early Therocephalians so far as known. Behind the ascending ramus of the dentary there is an opening between the angular and the sur-angular, and the anterior process of the angular which articulates with the dentary and the splenial is relatively slender. There is a narrow but rather stout process of the angular passing up to articulate with the sur-angular, and behind this is the upper opening into the cavity that lies between the outer and inner plates of the angular, and which I believe lodged a large salivary gland. The inner plate of the angular is continuous with the upper process, and is closely articulated to the sur-angular. The outer part forms a broad, fan-like, thin, corrugated bony plate. It has three depressions and four radiating ridges. Its lower border is very thin, and the greater part of the plate doubtless formed a protection for the gland.

The sur-angular is a well-developed curved bone which forms the upper part of the back third of the jaw. It articulates in front with the dentary, and doubtless also with the coronoid. Behind it passes down inside the angular and articulates with the articular.

The articular is a small, broad bone which forms a wide articulation with the quadrate.

On the back part of the palate is preserved much of the hyoid apparatus. As the hyoid apparatus has usually no articulation with the skull, or only a cartilaginous one, it is hardly to be wondered at that the palaeontologist knows little of fossil hyoids. Where perfect skeletons like those of a Pterodactyls or Ichthyosaurs are preserved, frequently the hyoids or some elements of the apparatus are seen, but in most fossil skeletons it is very exceptional to find any trace of the apparatus. I must have handled between 300 and 400 good Anomodont skulls, but I have never seen a hyoid in any of them, and so far as I know in only two specimens of Therapsids has any hyoid element hitherto been observed. I discovered remains of the apparatus in a British Museum specimen of *Thrinaxodon liorhinus*, and PARRINGTON has discovered the hyoid

in a specimen of *Galesaurus planiceps*. In each case it is represented by a pair of slightly curved rods.

In *Hofmeyria* the hyoid apparatus is present as a pair of curved rods with an imperfectly ossified structure connecting their anterior ends and a pair of very small rod-like elements behind the median cross-bar. I have figured the apparatus as it is seen, and given a restoration, figs. 112 and 122, Plate 8.

Opinions may differ as to the interpretation of these elements. When I first described the hyoid of *Thrinaxodon* in my book, "The Origin of the Human Skeleton" (1930), I was inclined to regard the two bony rods as homologous with the mammalian ceratohyal, and a comparison with the condition in the early marsupial *Trichosurus* seemed to render this probable. But I now think it more probable that the bony rods in *Hofmeyria*, *Thrinaxodon*, and *Galesaurus* are, as in the birds and chelonians, really the cerato-branchials or thyrohyals and represent the third visceral arch and not the second. The conclusion to which I came was partly due to the figures of the hyoids of marsupials and *Echidna* given by FLOWER (1870). In the hyoids of the kangaroo and wombat there figured, the ceratohyal is well developed and looks as if it might have been evolved from a much longer rod which had become reduced, while in *Echidna* the thyrohyal is represented as a short, bony element. Not feeling quite satisfied with my conclusion, I made preparations of the hyobranchial apparatus in both *Echidna* and *Ornithorhynchus*, and found that not only FLOWER's figure but also GÖPPERT's figure of *Echidna* are by no means satisfactory. The figure given by WIEDERSHEIM (1898) is much better, but not wholly right. The ossified part of the thyrohyal in *Echidna* gives a most misleading idea of the element, as it is continued far back as a cartilaginous rod, and it seems not improbable that this is the homologue of the bony rods in *Hofmeyria*, *Thrinaxodon*, and *Galesaurus*. The uniting bar is doubtless the basihyal, and the two small posterior elements are probably the representatives of the fourth visceral arches.

The hyobranchial apparatus in the typical mammal differs markedly from that in the typical Sauropsid. But if we assume that the early reptiles had an apparatus made up of a cartilaginous hyoid arch, a basihyal possibly ossified, a long rod-like ossified ceratobranchial, and a series of probably three other imperfect arches, we should have an ancestral type from which all the higher types might have arisen. In the higher reptiles and birds where the tongues have little more than backward and forward movements, the hyoid arch became reduced. In the mammals where the tongue became an organ largely for assisting in mastication, the hyoid became highly developed and specialized, and the posterior elements became reduced.

Though not a part of the skull, reference may be made to the upper two cervical vertebrae which are seen in the specimen. The proatlas is well-developed as a broad, short, curved plate. Behind it on the left side is seen the half-arch of the atlas, and on the right side it is even better preserved. This atlantal arch is essentially similar to that of the Anomodonts. On the right side of the specimen there is a delicate bony rod below the transverse process of the atlas. This, I believe, to be the atlantal rib. It passes round, and the lower end approaches close to the posterior end

of the ceratobranchial, and it might readily be regarded as an additional bony element of the third visceral arch. But it seems to me more probable that it is the slightly displaced atlantal rib.

IX—THE SKULL OF *Hyenosaurus platyceps*, BROOM

The type and only known specimen of this interesting Therocephalian is in the collection of the Transvaal Museum. The specimen has been in the Museum for many years, but there is no record of whence it came. Dr. v. HOEPEN examined the specimen, and had it to a great extent cleared of matrix, but as the specimen consists of only the back half of the skull, much of the palate, with the two jaws, and with the root of only one tooth, he evidently did not care to describe it or give it a name. Fortunately, the specimen, though so imperfect, is really very important, and reveals quite a lot of interesting points in the Therocephalian skull.

The skull is that of one of the later Therocephalians, and probably belongs to the *Whaitsidae*. Its nearest ally seems to be *Notosollasia*. I think we can safely assume that it came from Middle or Upper *Cistecephalus* beds. When complete, the skull must have measured about 270 mm in length, and the width across the squamosals is 216 mm. The posterior third of the skull is beautifully preserved, except for the upper temporal, and part of the basi-occipital regions, and each jaw is nearly perfect, except for the front of the symphyseal region which is missing in both.

The skull is characterized by the great size and width of the temporal fossae, by a low, very broad occiput, and by the powerful dentaries which are devoid of molars, while the angular part of the jaw is strongly developed but relatively short.

The occiput, as will be seen from the figures given, is exceptionally broad as compared with the height, and it differs in a number of respects from those of *Lycedops* and *Hofmeyria*, and also in many ways from that of *Whaitsia*. The upper part of the middle region is formed by the parietals, which form considerably more than a third of the crest of the occiput proper. The interparietal is relatively small and situated about midway between the crest and the foramen magnum. The tabulars are very large, and together form about half of the whole occipital surface proper. Each articulates on the inner side with the parietal, the interparietal, and the supra-occipital. Externally and inferiorly each articulates with the large squamosal and the supra-occipital, but in no place does it articulate with the opisthotic, or even approach it. BOONSTRA figures the occiputs of *Whaitsia* and *Notosollasia* with the tabular, giving a large articulation to the opisthotic outside and below the occipito-temporal opening. This is incorrect, the outer part of what he regards as the tabular is really part of the squamosal. The tabular does not appear on the anterior side of the occipital plate.

The supra-occipital is relatively small but fairly wide. It meets the squamosal above the occipital opening. The exoccipital is partly preserved on the right side. It agrees with the exoccipitals in other Therocephalians.

The squamosal is a very large bone, and it is almost perfectly preserved on the left side, and nearly complete on the right side. As in apparently all Therocephalians, it forms part of the true occipital surface, and here as in other higher types it forms a large part. The squamosal might be described as a large bone whose central part is moulded round the front of the paroccipital process of the opisthotic. When viewed from the front the squamosal entirely hides the outer part of the opisthotic. Its anterior inner side forms a long articulation with the parietal, and its inner lower corner articulates with part of the opisthotic, and a process passes inwards and forwards to articulate with the prootic and the pterygoid. Anteriorly, the squamosal forms much of the temporal arch and rests on the posterior process of the jugal. Inferiorly, the squamosal articulates with the quadrate and the quadratojugal; and a posterior ridge caps the outer end of the opisthotic. On the outer side of this ridge is a marked groove, which leads down towards the inner end of the quadrate, and as in *Lycedops* doubtless ended at a tympanic membrane that was attached to the lower edge of the squamosal and the quadrate.

The quadrate is fairly large. In the specimen it may have been displaced a little inwards. The quadratojugal is a small triangular bone which has been apparently more firmly attached to the squamosal than to the quadrate, as with the apparent lateral displacement of the quadrate the quadratojugal has retained its squamosal articulation. The quadrate has a long, irregularly cylindrical, articular surface, 40 mm in length. There is a well-developed ascending process 13 mm from the inner end of the quadrate. This inner end doubtless gave articulation to the outer end of the stapes as in other Therocephalians, but the stapes has been lost from this specimen. If the quadrate be shifted to the original position about 10 mm farther out, the ascending process which is seen would doubtless be in a pocket in the lower part of the squamosal, and probably the tympanic membrane was attached to the inner border of this ascending process.

The opisthotic forms, as in typical Therapsids, a well-marked paroccipital process, but it differs from that of the lower Therocephalians in having only a single, irregularly oval, outer end. This paroccipital process forms the lower wall of the occipitotemporal opening. The upper part of this process has a large articulation with the squamosal as in *Whaitsia*. The inner end of the opisthotic has similar relations to the supra-occipital and prootic seen in the earlier Therocephalians.

As the posterior part of the basi-occipital is lost with the whole of the left exoccipital and much of the right exoccipital no satisfactory description can be given of the relations to the exoccipital. The occipital condyle must have been relatively small.

There are well-marked processes on the base of the skull formed by the basi-occipital, opisthotic, and prootic, and supported by the vomer, on the base of which are the fenestrae ovals. The processes are shorter than in the early Therocephalians. The prootic is moderately large, and so far as can be seen fairly similar to that of other Therocephalians. In front and partly hidden by the epipterygoid is a small pleuro- or latero-sphenoid, which appears to have a foramen at its base, presumably for the sixth nerve.

The vomer is a relatively short triangular bone. Its posterior end forms supports in front of the processes in which are the fenestrae ovals. In the middle line the vomer passes back some distance under the basi-occipital. In a median section of the base of the brain-case a well-marked suture can be traced between the basi-occipital and the basi-sphenoid; and it is extremely interesting to note that the basi-sphenoid is a well-ossified and distinct cartilage bone that lies exactly as in mammals in front of the basi-occipital. The vomer is quite free from at least most of the base of the basi-sphenoid, a clear suture being quite apparent between the bones. It appears as if the vomer articulates with not only the base of the basi-sphenoid but also with part of the front as shown in the figure; but owing to the condition of the bone it is difficult to be sure whether or not there is an ankylosis in any part between the bones. The vomer has a large, very deep median plate which passes forwards between the pterygoids. Its upper anterior portion is grooved for the lodgement of the inter-orbital cartilage. The full depth of the median plate cannot be determined, but it is certainly large. The shaded patches shown in the drawing, fig. 127, Plate 9, which are drawn from preserved portions of the plate, give a fair idea of its extent. Outer plates of the vomer clasp the posterior pterygoid plates on their outer sides, as shown in the section figured (fig. 126, Plate 9).

The pterygoids are almost perfectly preserved. Quite manifestly the pterygoid forms the back part of the larger outer process, as in all Therocephalians in which the parts are satisfactorily preserved. BOONSTRA (1934) figures the transpalatine as forming the back of the process in *Notosollasia*, *Theriognathus*, and *Whaitsia*. In the figures I gave of the palates of *Notosollasia* and *Whaitsia* in 1932, I indicated the pterygoids as forming the back of the processes. The fact that the pterygoid certainly forms the back of the process in *Hyenosaurus* seems to render it probable that my interpretation of the condition in *Whaitsia* and *Notosollasia* is correct. There is no sub-orbital vacuity. Posteriorly, the pterygoid has a long outer process which extends to very near the quadrate—possibly articulating with it.

Above the back part of the pterygoid is seen the large epipterygoid. This has a short, very broad, upper portion, and a long, flat base which extends outwards and backwards on the top of the outer process of the pterygoid; but it ends a considerable distance short of the quadrate.

The transpalatine (ectopterygoid) is relatively large. It forms the front of the outer part of the pterygoid process. It extends well forward on the outer side of the palatine. Internally, it has a long articulation with both the palatine and the pterygoid.

The palatines are very well developed. Along the inner side of the maxillo-palatine suture there is a broad development of thickened bone as if the palatine bone in the absence of molar teeth shared in mastication. On passing forward towards the internal nares the palate is deeply excavated. The prevomers clearly have large palatal plates, but the exact sutures between these plates and the palatines and pterygoids cannot be made out. Between the internal nares the prevomers are in the posterior part narrow. Most probably they broaden out in front.

Much of the parietals is preserved. The two bones are completely fused. There has manifestly been a narrow intertemporal crest, though most of it is missing. So far as preserved, there is no pineal foramen, though one may have been present. Posteriorly the parietals spread out on the back wall of the very large temporal fossae and articulate with the squamosals. Superiorly the parietals form the middle part of the temporal crest.

Each mandible is practically complete, and the detailed structure of nearly every bone can be determined.

The dentaries are large bones which form about three-quarters of the whole jaw. In neither of the dentaries are any molars present, though in each dentary the portion of the bone in which we should look for molars is satisfactorily preserved. In the left dentary we have the root of a fair-sized canine, whose section measures 15 mm by 10 mm. A very small part of the very front of the dentary is missing. So far as preserved there is no trace of incisors. Judging by the curves it seems probable that only 15 mm of the bone are gone, and the incisors must have been small. In the right jaw there is no evidence of either incisors or molars, and there is no trace of a canine. The absence of a canine in the right dentary and the general massiveness of the bone in association with the huge fossae for the temporal muscles is very remarkable. The coronoid process is very short, only rising a short distance above the sur-angular. The symphysis has evidently had only a very loose membranous attachment.

The splenial is a fairly large bone lying on the inner side of the lower portion of the front half of the jaw. It does not quite reach the lower margin of the dentary. Posteriorly, the splenial meets the front of the coronoid, and also articulates with the pre-articular and the angular.

The coronoid is fairly well preserved in the right mandible. When the specimen was developed twenty years ago, the coloured preparator, though he did most excellent work, cleared the dentary a little too far back before he observed the thin coronoid. The coronoid is a crescent-shaped bone whose upper part rests on the dentary and the sur-angular, and whose lower part rests on the pre-articular and the angular, and also articulates with the splenial.

The back part of the jaw is formed, as in other Therapsids, by the articular, the angular, the sur-angular, and the pre-articular.

The articular is a short, wide bone whose articular face is transversely grooved to hinge on the broad quadrate. Much of the outer side is hidden by the sur-angular and angular; while the greater part of the inner side is hidden by the pre-articular. The short anterior part of the bone is firmly fixed between the pre-articular and the angular.

The sur-angular is a short but exceptionally strong bone. The anterior two-thirds of the bone are closely articulated to the coronoid portion of the dentary, and overlying the anterior end is part of the coronoid bone. The back part of the bone is closely articulated to the articular, and much of the outer side is covered by the angular.

The angular is relatively smaller than in most Therocephalians. The anterior end passes forward between the splenial and the dentary. Part of it is overlain by the coronoid bone, and the greater part of the inner side covered by the pre-articular. The outer side of the bone has a relatively small outer plate, which is unlike that of the earlier Therocephalians, but recalls in some respects the outer plate of the angular in the Titanosuchids. There is an elongated opening above it which is continued round to the narrow opening on the lower margin of the jaw. There seems to be a notch on the lower border of the bone, but how much of this is due to imperfection cannot be determined, probably only a little.

The pre-articular has a well-developed posterior end which clasps the outer side of the articular. The anterior portion of the bone is a comparatively thin plate which lies on the angular and passes forward under the coronoid and splenial.

Hyenosaurus is clearly one of the later Therocephalians. It differs from *Whaitsia* and *Notosollasia* in that the occiput recedes very markedly, so that no part of it can be seen from directly above. The structure of the angular also differs very considerably from that of any of the previously known later Therocephalians.

X—SUMMARY AND CONCLUSIONS

The Therocephalians first appear in South Africa at the base of the *Tapinocephalus* zone, which may perhaps be regarded as Middle Permian. It is unfortunate that the Eccles beds below the *Tapinocephalus* zone are extremely poor in vertebrate fossils, and the few that are known are too imperfect to throw much light on the origin of the types that appear in the *Tapinocephalus* beds. In the lowest beds of the *Tapinocephalus* zone we find a rich fauna. We have a number of species of Pareiasaurians belonging to the genus *Bradysaurus*, the primitive pro-Therapsid *Anningia*, various Therocephalians, various Gorgonopsians and already Anomodonts and a large number of Dinocephalians, both the herbivorous Tapinocephalids and the carnivorous Titanosuchids. We thus know nothing of the common ancestors of the Dinocephalians, the Therocephalians, the Gorgonopsians, and the Anomodonts.

While we thus know nothing of the direct ancestors of the Therocephalians, the group is of the greatest interest, as there is no reasonable doubt that the Scaloposaurids of the Lower Trias are descended from the earlier Therocephalians, and also little doubt that the Middle or Upper Triassic Bauriamorphs are descended from a Scaloposaurid ancestor. Further, it seems probable that the Rhoetic Ictidosaurians arose from a Bauriamorph, and highly probable that the first mammal arose from an Ictidosaurian. There is thus much probability that the line of mammalian descent passed through some members of the Therocephalia, and thus the group as probably containing our own remote ancestors seems worthy of very considerable study.

The skull, as we have seen, is remarkably mammal-like in general shape and structure. The temporal arch is formed as in the mammal by the jugal and the

squamosal. There have manifestly been large temporal muscles which were attached to a coronoid process of the dentaries as in mammals. The teeth already were specialized as incisors, canines, and molars.

On the other hand, there are many striking points of difference. The palate is very unlike that of any mammal. Even if the mammal had no secondary palate the difference between the mammalian and Therocephalian type would be striking. The mammal has a large median vomer situated, in most, well forward and generally extending to near the front of the snout. The Therocephalian has a pair of bones in front of the palate, which I consider are homologous with the dumb-bell-shaped bone of *Ornithorhynchus*, and at the back of the palate is a median bone—the para-sphenoid of most authors—a bone which does not occur in mammals. For many years I have maintained that the reptilian “para-sphenoid” evolved into the mammalian vomer, and the facts now known about the structure in the Therocephalians, the Gorgonopsians, the Anomodonts, and the Ictidosaurians seem to me to confirm this conclusion.

Then we have the lower jaw in the Therocephalians with the posterior third formed mainly by the sur-angular, angular, pre-articular, and articular bones, and the articular hinging on a well-developed quadrate. Further, in the Therocephalian skull we have many bones which are completely lost in the mammal. And yet I think it can be shown how in nearly every feature we have pro-mammalian characters whose evolution into the mammalian condition can be readily understood.

Some of the more important problems raised by the study of the Therocephalian skull may be considered in some little detail.

Dental Succession

In the mammal we have typically a first set of teeth comprising incisors, a canine, and four molars. These are shed and replaced by a second set which consist of incisors, a canine, four replacing molars and three further molars which have no antecedents. In some Cynodonts we have a succession of teeth almost typically mammalian. Certainly a number of anterior molars are replaced. In the Gorgonopsians we have a dental succession in the incisors and the canine, but apparently not in the molars. In the Therocephalians we have certainly a dental succession in the incisors and canines. Whether there are more than two sets is at present unknown. As the succession in the incisors seems to be irregular, it seems probable that there is an indefinite succession of the incisors. But it seems possible that there are only two sets of canines. And so far as the evidence goes there is no satisfactory proof of a dental succession in the molars. The fact that in *Hofmeyria* the molars are not all equally developed suggests that in this later Therocephalian there may be a dental succession. In the *Whaitsidae* it is probable that there is a set of molar teeth that became lost later on and are not replaced. In the only known skull, *Lycideops longiceps*, which is certainly immature, there is a series of small molars which will probably be shed, and possibly not replaced.

Very probably when more specimens are examined we shall find that in earlier Therocephalians there are three or more sets of incisors, two sets of canines, and only one set of molars.

Prevomer and Vomer

When in 1895 (Broom, 1900) I was studying the comparative anatomy of the Organ of Jacobson, I was led to the conclusion that the lacertilian so-called "vomer" is really the homologue of the dumb-bell bone in the platypus, and not of the mammalian vomer, and I proposed for it the name of "Prevomer". This raised the further question of what bone if any in the lizard is the homologue of the mammalian vomer. When one examines a very young lizard's skull one finds a median membrane bone extending forward from the basi-sphenoid region under the interorbital septum in much the same way as the mammalian vomer passes forward under the nasal septum; and it is hard to resist the idea that this median bone in the lizard, which has been called the "para-sphenoid", will ultimately prove to be the real homologue of the mammalian vomer. This view was first suggested by BLAND SUTTON in 1884.

Owing to the wide difference in structure between the lizard and the mammal, and the complete absence of any living form that in any way helps to bridge the gulf, it has been very difficult to prove that the reptilian so-called "para-sphenoid" is really the mammalian vomer.

For the last 36 years I have been engaged in the study of the fossil mammal-like reptiles of the Permian and Triassic beds of South Africa, and one of the problems that has never been forgotten is whether or not the "para-sphenoid" of the reptile becomes the vomer in the mammal. Of course, the paleontologist has rarely a chance of studying any but adult specimens, and even in good adult specimens it is often very difficult to determine the limit of bones, especially where the specimens are a little crushed and the matrix hard. In fact, it has only been possible to unravel the structure of the base of the skull in the mammal-like reptiles by having sections made.

It is only quite recently that we have known satisfactorily the structure of the base of the skull in the Therocephalians, the Gorgonopsians, the Anomodonts, and the Cynodonts. We also know something of the base of the skull in the Dinocephalians, the Bauriamorphs, and the Ictidosaurians.

In the Dinocephalians, as exemplified by *Dinophoneus*, we find a pair of large prevomers in the front of the palate, and a small median vomer or para-sphenoid in the basi-sphenoid region, and probably extending some distance in front of the basi-sphenoid. The exact condition and size of the basi-sphenoid are unknown, and it is also unknown whether this vomer or para-sphenoid is completely fused with the basi-sphenoid.

In the early Therocephalians, as we have just seen, there are a pair of prevomers in the front of the palate which are always distinct. In the basi-sphenoid region there is a fairly large median vomer or para-sphenoid. It extends well back under much of the basi-occipital, and sends a process forwards, supporting the cartilaginous inter-orbital septum. It would appear from the sections that the basi-sphenoid is quite a

small element which is anchylozed to part of the top of the vomer, and articulates with the basi-occipital.

In *Hyenosaurus* the vomer differs considerably from that in *Lycedops*. The basi-sphenoid is here a large, well-ossified element which, as in mammals, is articulated to the front of the basi-occipital by a well-marked suture. Below it is a deep median element, which is clearly separated by a suture from the posterior half of the basi-sphenoid, but is less clearly separated from the anterior half. This large median vomer has a posterior plate which passes back for some distance below the basi-occipital. In front the vomer clasps the inner plates of the pterygoids, the two plates being firmly articulated between the three anterior plates of the vomer. Laterally, the vomer has plates which pass in front of the auditory tubera. And superiorly the vomer has a groove for the interorbital cartilage. In the Gorgonopsians the prevomers are always fused into a median bone between the internal nares. The true vomer or para-sphenoid is a median bone which bears some resemblance to that in the early Therocephalians. It differs in having a smaller descending median pharyngeal plate. The relations to the other elements of the basis cranii are only known fully in the immature *Cynarioides*, and here the basi-sphenoid is not yet ossified. Manifestly the vomer is entirely or nearly wholly a membrane bone which passes back for some distance under the basi-occipital.

In some Bauriamorphs, e.g., *Bauria*, the palatal condition is probably very similar to that in the Therocephalians, except that there is a secondary palate formed.

In the Cynodonts there is in the front of the palate a median bone largely hidden by the secondary palate. This is the fused prevomers. In the back part of the palate is a median vomer which passes back a little way below the basi-occipital, and in front passes forward as a long, narrow process which supports the inter-orbital median cartilage. I have been unable to determine how much of the upper part of the bone is basi-sphenoid—probably only a small part.

In the Anomodonts the vomerine condition differs considerably from that in the carnivorous Therapsids. The prevomers are fused to form a deep median plate which lies between the internal nares. Immediately above the fused prevomers is a long trough-like vomerine spur which for a time was believed to be part of the prevomers, but is really quite distinct. This is manifestly a typical "para-sphenoid". It is also nearly as manifestly a typical vomer. It is completely fused with the large basi-sphenoid behind, as is the vomer in *Ornithorhynchus*. If it was not anchylozed to the basi-sphenoid it would exactly agree with the vomer of higher mammals.

In the Ictidosaurians the palatal condition is imperfectly known. There is, however, undoubtedly a large median vomer which is well forward and separates the pterygoids. So far as is known it is almost typically a mammalian vomer.

Then, as I have elsewhere pointed out, in *Ornithorhynchus* we have a pair of prevomers in the front of the palate to form the dumb-bell-shaped bone, and behind the dumb-bell bone there is a long typical vomer which is remarkable in being very early fused with the basi-sphenoid, and to be thus exactly like the "para-sphenoid" of the reptiles such as *Ichthyosaurus* or the lizards.

There can thus hardly be a doubt that the dumb-bell bone of *Ornithorhynchus* is the homologue of the paired vomers of the reptiles; and the mammalian vomer when traced down proves to be the homologue of the bone which has been called the "para-sphenoid".

Pre-sphenoid and Basi-sphenoid

In the Anomodonts the basi-sphenoid is always a large bone which articulates with the basi-occipital, and it is typically mammalian in its relations. There can thus be no doubt about its homology. Some distance in front of the basi-sphenoid is another median cartilage bone. It articulates with the frontals in front. Lateral canals in this bone appear to have lodged the olfactory lobes of the brain, and in the lower part is a deep sulcus in which probably lay the optic commissure. This bone has given rise to considerable dispute as to its homology. It has been called ethmoid, mesethmoid, sphenethmoid, pre-sphenoid, and even orbito-sphenoid.

A comparison with the condition in the young *Ornithorhynchus* seems to prove that this bone must be not the mesethmoid but the mammalian pre-sphenoid. The bone in the *Ornithorhynchus* supports the two orbito-sphenoids exactly as does the pre-sphenoid in the higher mammals. Assuming it to be the pre-sphenoid, there is then no mesethmoid in any of the lower reptiles, while in most this pre-sphenoid can be traced.

In Gorgonopsians this pre-sphenoid is always present and well ossified. In the Therocephalians it is probably always present, but as it is feebly ossified and loosely attached to the frontals, it has only been found *in situ* in one specimen. In the Cynodonts a fully ossified pre-sphenoid is not yet known. There is little doubt one will be found in the larger forms. In the sections I have made of *Thrinaxodon liorhinus* the pre-sphenoid is not fully ossified, but there are clear indications of the element probably of partly calcified cartilage. In position it agrees with the bone in the Therocephalians. It is present as a well-developed bone in *Bauria*.

The basi-sphenoid is probably present in all Therapsids, but it is difficult in most forms to say how much of the conjoined bone is basi-sphenoid and how much vomer or para-sphenoid. In the Anomodonts most of the bone is manifestly basi-sphenoid. In the Gorgonopsians and Therocephalians most of the bone appears to be vomer. In the Therocephalians and probably in the Cynodonts the basi-sphenoid only forms a small part of the conjoined bone.

Pleuro-sphenoids

When studying the development of the Marsupial skull in 1898 I was surprised to find that the alisphenoid develops from a little cartilage which lies well outside the cranial wall, and has apparently nothing to do with it. Its morphological relations seemed very similar to those of the rudimentary epipterygoid in the lizard *Agama*, and soon I came to the conclusion that the alisphenoid is probably the homologue of the lacertilian epipterygoid.

During the early years of the present century we came to know something of the epipterygoid in the more important groups of Therapsids. In the Anomodonts we found an almost lizard-like epipterygoid or columella, and in the Therocephalians an epipterygoid rather broader. In the Cynodonts what was manifestly the same element was seen to be broadened out into a fan-like bone which one can hardly doubt is an alisphenoid. When more types were studied, and when the relations of the bones to blood vessels and nerves were examined, in living lizards and other reptiles, it became more and more manifest that the alisphenoid of the mammals is the homologue of the epipterygoid of the lizard. And now it is agreed by practically all morphologists that this is so. GOODRICH, in his recent book, "Structure and Development of Vertebrates", states that "there is little doubt that BROOM's contention that the mammalian alisphenoids are derived from the epipterygoids of lower Tetrapods is correct".

But another question arises. In the crocodile and bird there is a bone usually called "alisphenoid", which is really part of the cranial wall. It thus cannot be the homologue of the mammalian alisphenoid if the alisphenoid is homologous with the lizard epipterygoid. Further, in some reptiles, *e.g.*, the *Phytosaurus*, there is both an epipterygoid and a bone which is clearly the homologue of the so-called "alisphenoid" of the crocodile. When the nerve relations of the bones are examined we seemed forced to the conclusion that this so-called "alisphenoid" is really homologous with the cartilage in the lizard skull called the *pila antotica*. This is a pillar of cartilage which passes up from the front of the prootic cartilage to meet the lateral cartilaginous wall—the *taenia marginalis*. Its base is pierced by a foramen for the sixth nerve, at least in *Lacerta*. It probably represents part of the original cranial wall. When ossified, it seems worthy of a special name, and various names have been suggested—sphenolateral (GAUPP), oto-sphenoid (BROOM), latero-sphenoid (GREGORY and NOBLE), and pleuro-sphenoid (VAN WIJHE). GOODRICH prefers the latter form. As a cartilage it can be traced from fishes, through most amphibians and reptiles to the monotremes. It is rarely ossified. GOODRICH suggests that the large "sphenethmoid" of some Stegocephalians may include this posterior pleuro-sphenoid, and with this I agree.

In the *Phytosaur Machaeropsopus gregorii*, as shown by CAMP (1935), the pleuro-sphenoid (latero-sphenoid) is a large bone with a foramen at its base for, it is believed, the sixth nerve and certain branches of the fifth. The upper part of the bone forms a large part of the wall of the brain-case, and it passes forwards to form the inter-orbital septum and the wall of the olfactory lobes (what I believe to be the pre-sphenoid and most others call the sphenethmoid). Very probably if a young *Phytosaur* were examined we should find the pleuro-sphenoid and the pre-sphenoid distinct bones. The fact that the two elements are fused in the *Phytosaurs*, and also in the Embolomorous amphibians seems strongly to support the view that they are ossifications of the same lateral wall cartilage.

In the Therapsids, so far as is known, we only find an ossified pleuro-sphenoid in the Therocephalians, the Bauriamorphs, and some Gorgonopsians. It apparently never occurs in Anomodonts or Cynodonts as a bone. In the Therocephalians, which I

have sectioned, it is a somewhat rudimentary structure; but whether in the ancestors of the Therapsids it ever was a large bone is very doubtful. Probably in most early reptiles it was present as a cartilage, while in only a few did it become a well-developed bone. Near the base of the bone in the Therocephalians is a foramen possibly for the sixth nerve. I cannot find any suture between the base of the bone and the prootic.

The presence of the ossified pleuro-sphenoid in the Therocephalians is interesting in view of the same element being present in the monotremes as the pila antotica.

Auditory Apparatus

Many have been the attempts that have been made to trace the probable steps by which the middle ear of mammals has been derived from the ear of reptiles. REICHERT's theory that the mammalian incus is the homologue of the reptilian quadrate, and the malleus the articular has in the last 100 years always had the support of many leading morphologists. A number of other theories have from time to time been suggested, but with the steady advance of palaeontology and comparative anatomy the evidence in favour of REICHERT's view has become more and more convincing. GAUPP's advocacy of the view with his wealth of embryological evidence which he published in his book, "Die Reichertsche Theorie" (1913), has completely convinced the scientific world of the main truth of the theory. But we are still in considerable perplexity as to how the change in the hinge of the jaw took place, and how the old hinge bones came into the ear, and where the tympanic membrane was during the changes.

In 1912, in discussing the auditory region in *Dicynodon*, I gave a series of diagrams illustrating what may have been the changes from a condition such as that seen in *Dicynodon* to the mammalian condition. I assumed as probable that, with the dentary articulating with the squamosal, the angular, articular, and quadrate became steadily reduced, and the angular and articular, becoming free from the jaw, shifted a little back and developed into the tympanic and malleus. I assumed that the tympanic membrane always was situated in the earlier stages behind the quadrate and near the outer end of the stapes.

PALMER (1913), from the examination of the jaw in the foetal *Perameles* and the comparison of the back of the jaw with that of the Cynodonts, came to the conclusion that even in the Therapsids the angular had a curved process which probably supported part of the tympanic membrane. This suggestion has been supported by quite a number of morphologists, but has never appeared to me to be at all likely. In the first place, the curved process of the angular in Cynodonts is undoubtedly the homologue of the outer plate of the angular which we can trace up from the Dinocephalians, and we may be perfectly certain that this outer plate did not support the tympanic membrane in Dinocephalians, Therocephalians, Anomodonts, or Gorgonopsians. Then it seems impossible to conceive of the mandible opening widely and closing and with every movement carrying the tympanic membrane into a different position. But if these arguments were not convincing we now have the certainty that

in the Therocephalians at least the tympanic membrane was attached to the squamosal and the quadrate, and not in any way to the jaw. In fact, the position of the membrane in the Therocephalian is exactly where I assumed it must be, in my 1912 paper. We thus have a new definite step in the advancement of our knowledge.

The discovery of the extra-stapedial is a second and equally important advance. But it raises a new problem—how the extra-stapedial disappeared and had its place taken by a process of the articular. Unfortunately, the condition of the auditory apparatus is less satisfactorily known in the Anomodonts, the Gorgonopsians, and the Cynodonts than in the Therocephalians. All these groups have a fairly massive stapes as a rule, and so far no evidence of any extra-stapedial has been detected. Possibly a cartilaginous extra-stapedial has been present.

When the jaw had formed its new articulation between the dentary and the squamosal, the quadrate and the articular doubtless rapidly decreased in size, and the articular with its membrane-bone elements, the pre-articular on the one side and the angular on the other, became detached from the jaw, and no longer moved with the jaw. As the new hinge of the jaw was mainly outside of the middle ear the tympanic membrane became more deeply placed in the side of the head, and most probably the old articular came to support part of the membrane, and then with it the angular. The angular as a membrane bone became a more suitable support than either the quadrate (incus) or the articular (malleus), and soon it became the main support of the membrane and developed into the tympanic bone. Very probably the tensor tympani muscle (one of the muscles of the early jaw) played an important part in the transformation of the jaw elements into auditory ossicles.

How the extra-stapedial disappeared and its place was taken by a process of the articular (malleus) is more difficult to imagine. What has probably happened has been that the posterior end of the articular for a time supported the tympanic membrane in front. And then the angular gradually took its place and the articular (malleus) became shifted towards the middle of the membrane and the extra-columella became reduced and lost. If we ever get a good specimen of a skull of *Tritylodon*, or one of the other Upper Triassic mammals, with the ear region perfectly preserved we shall probably have a full solution of the question.

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XII—DESCRIPTION OF PLATES

Lettering

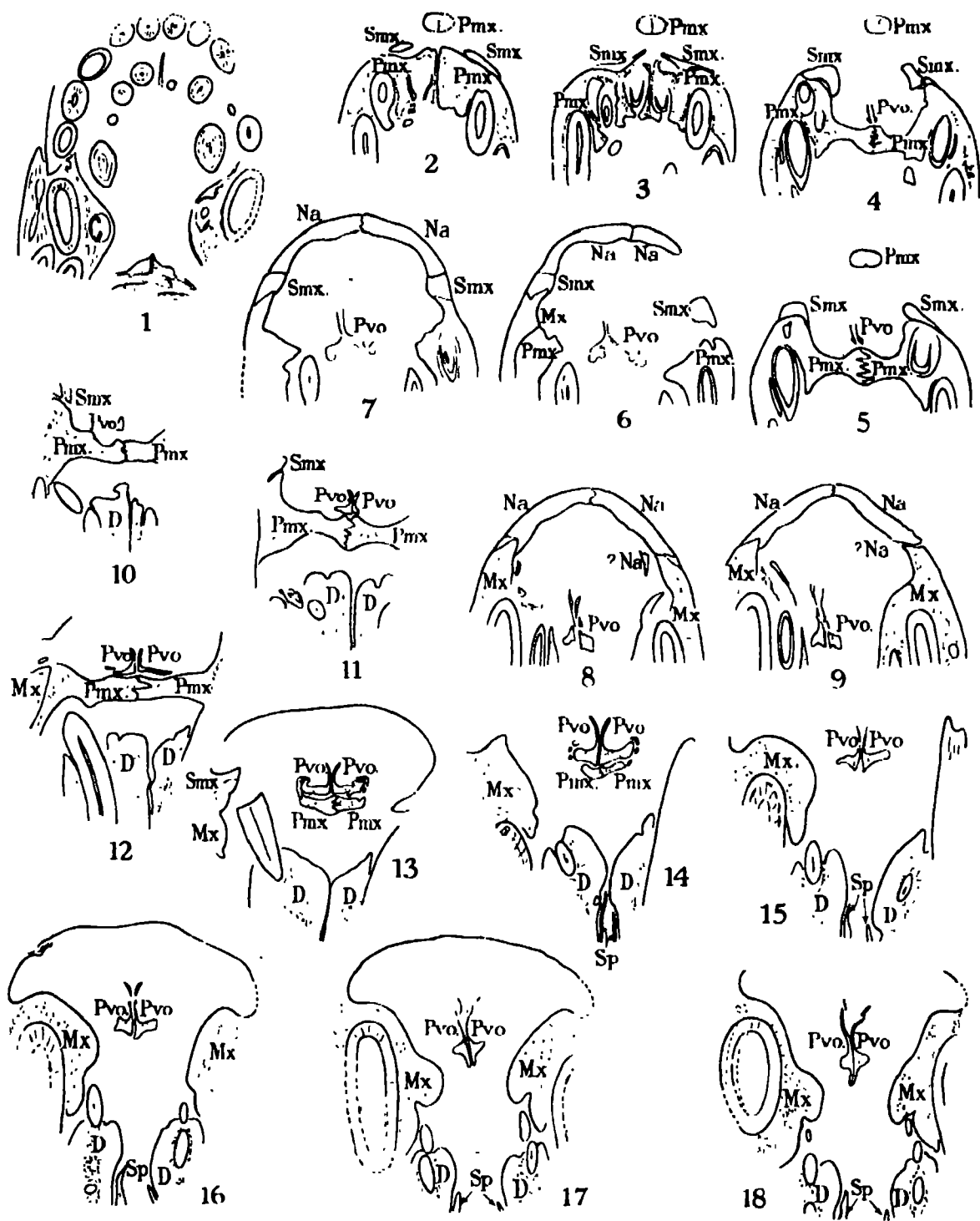
Ang., Angular ; *Art.*, Articular ; *At.*, Atlas ; *Ax.*, Axis ; *Bo.*, Basi-occipital ; *Bs.*, Basi-sphenoid ; *C.Ax.*, Centrum of Axis ; *Co.*, Coronoid ; *D.*, Dentary ; *Eo.*, Exoccipital ; *Ept.*, Epipterygoid ; *Fr.*, Frontal ; *Ip.*, Interparietal ; *Ju.*, Jugal ; *L.*, Lacrimal ; *Mx.*, Maxilla ; *Na.*, Nasal ; *Oo.*, Opisthotic (Paroccipital) ; *O.p.*, Odontoid process of the Axis ; *Pa.*, Parietal ; *Pal.*, Palatine ; *P.art.*, Pre-articular ; *P.at.*, Proatlas ; *Pf.*, Pre-frontal ; *Pls.*, Pleuro-sphenoid (Latero-sphenoid) ; *Pmx.*, Pre-maxilla ; *Po.f.*, Post-frontal ; *Po.o.*, postorbital ; *Pro.*, Prootic ; *Ps.*, Pre-sphenoid (Sphenethmoid) ; *Pt.*, Pterygoid ; *Pvo.*, Prevomer (Vomer) ; *Q.*, Quadrate ; *Qj.*, Quadratojugal ; *S.ang.*, Sur-angular ; *Smx.*, Septomaxilla ; *So.*, Supra-occipital ; *Sp.*, Splenial ; *Sq.*, Squamosal ; *St.*, Stapes ; *Tb.*, Trabecular ; *Trp.*, Transpalatine (Ectopterygoid) ; *Vo.*, Vomer (Para-sphenoid) ; XII., Twelfth nerve.

PLATE I

Fig. 1—Horizontal section of the snout of *Trochosaurus dirus*, Broom ; cutting through most of the teeth.
½ natural size.

Figs. 2-9—Transverse sections of the snout of *Trochosaurus dirus*, Broom. ½ natural size.

Figs. 10-18—Transverse sections of the snout of a moderately large Therocephalian, probably *Pristerognathus vanderbyli*, Broom. ½ natural size.

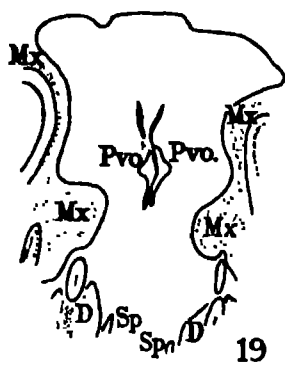


Trochosaurus dirus and *Pristerognathus vanderbyli*.

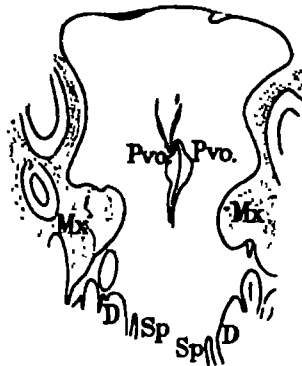
PLATE 2

Figs. 19-24—Transverse section of the snout of a moderately large Therocephalian, probably *Pristerognathus vanderbyli*, Broom. $\frac{1}{8}$ natural size.

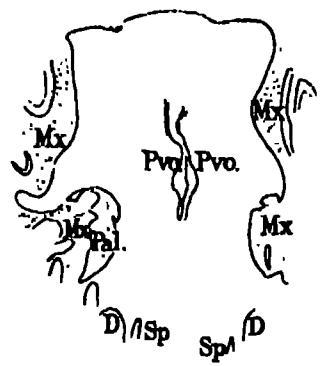
Figs. 25-32—Transverse sections of the skull of *Pristerognathus minor* (Haughton). $\frac{1}{8}$ natural size.



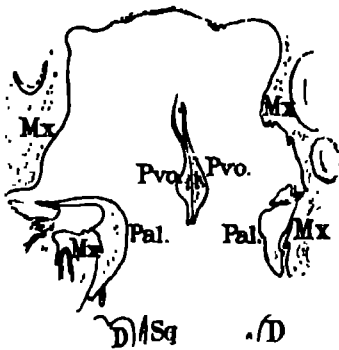
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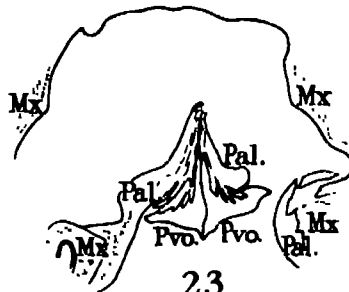
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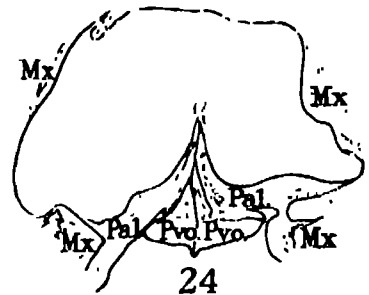
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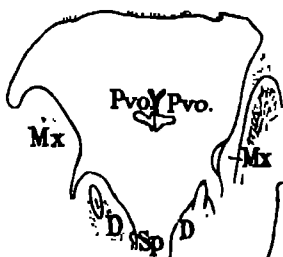
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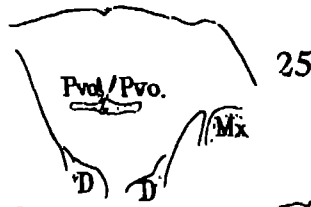
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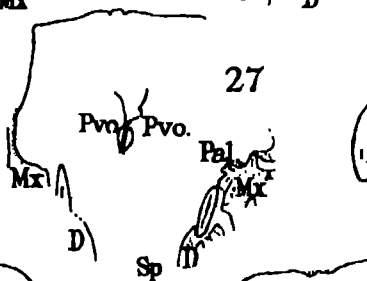
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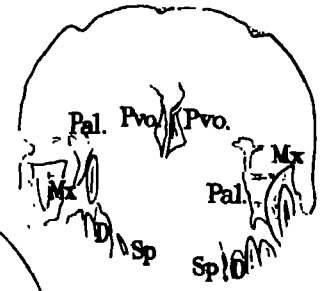
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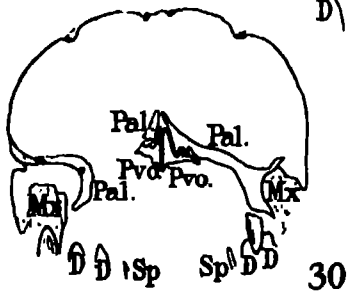
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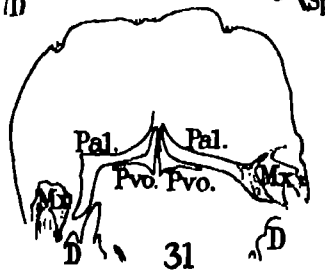
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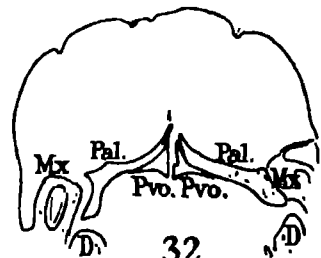


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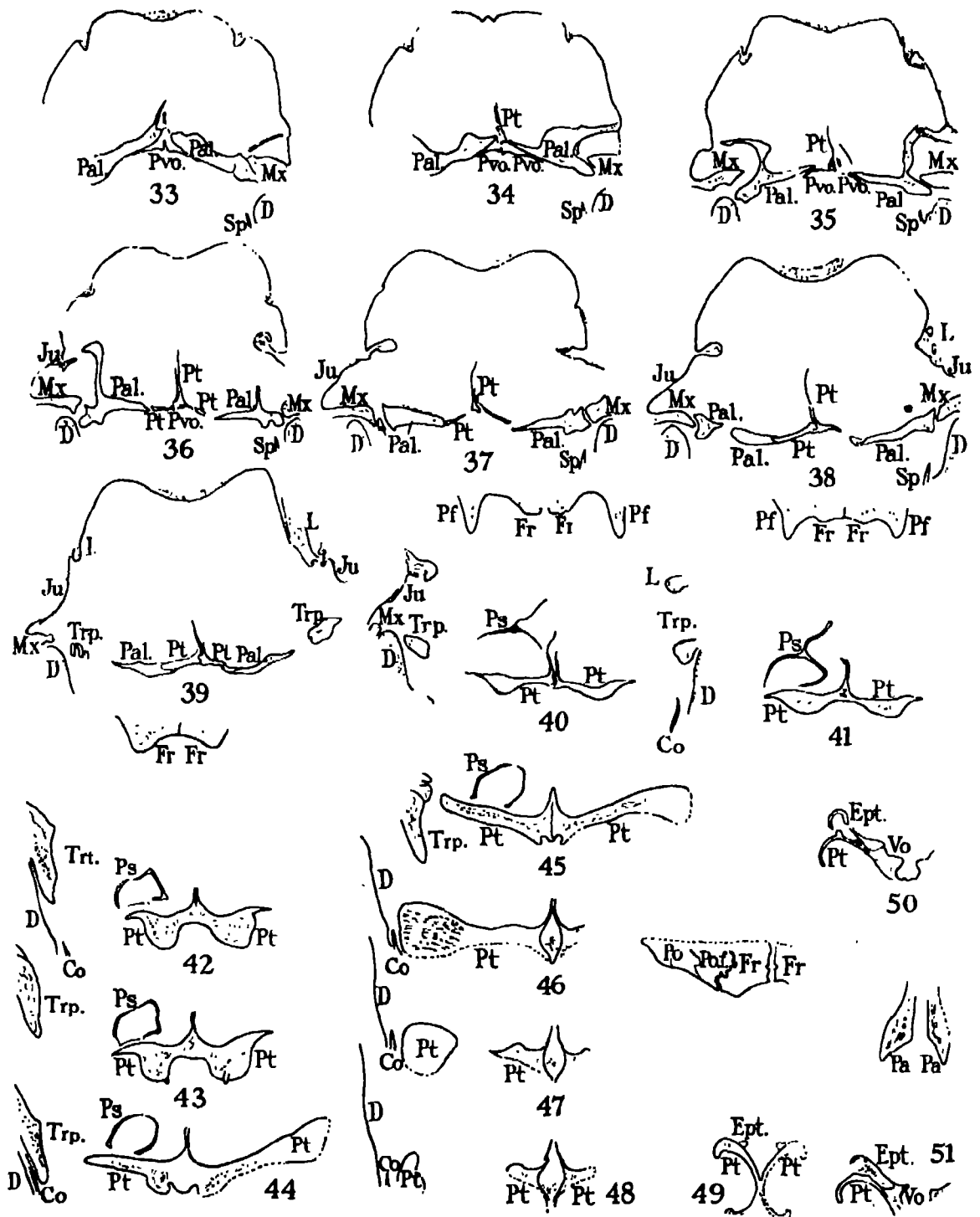


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Pristerognathus vanderbyli and *Pristerognathus minor*.

PLATE 3

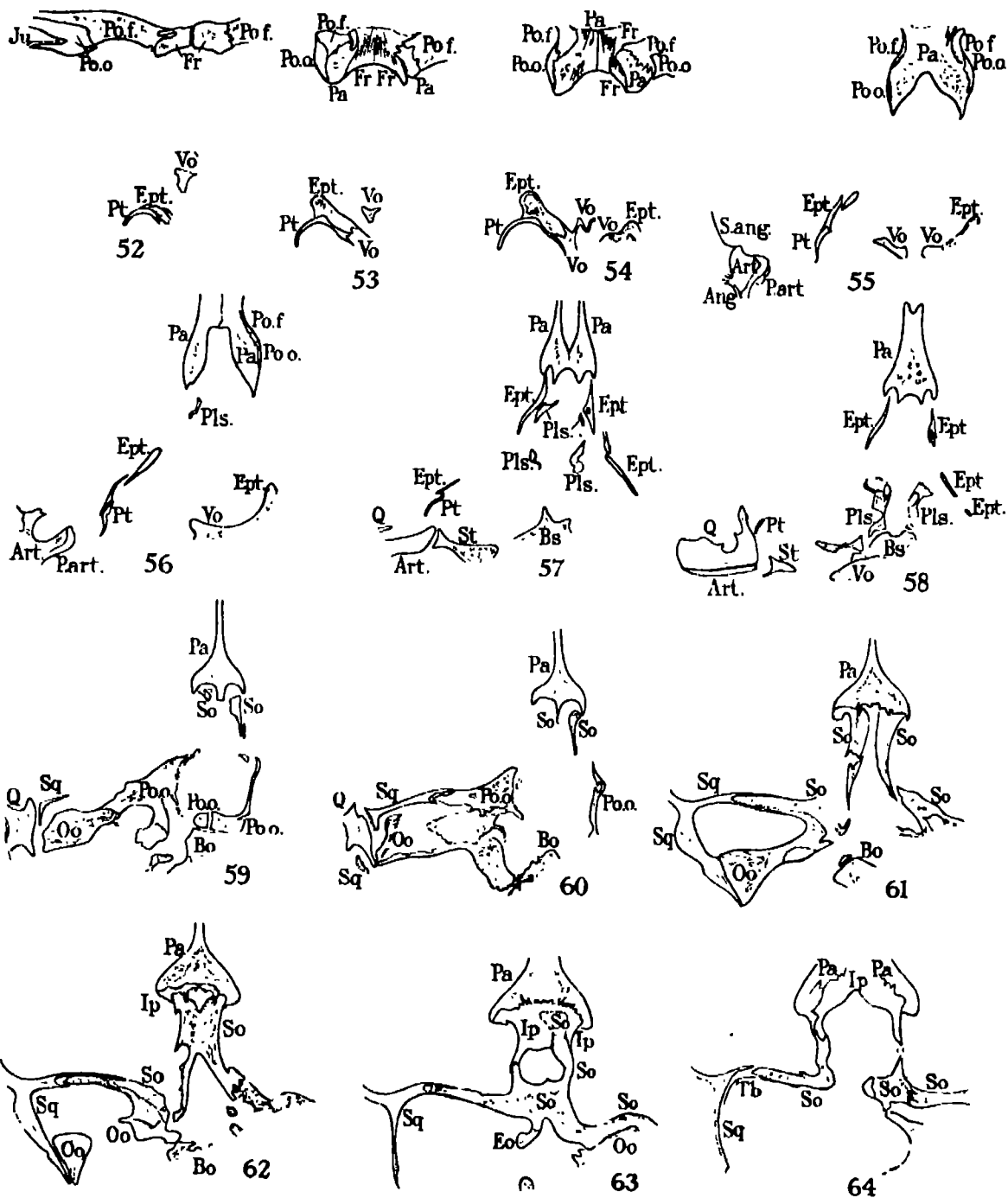
Figs. 33-51—Transverse sections of the skull of *Pristorognathus minor* (Haughton). $\frac{1}{2}$ natural size.



Pristerognathus minor.

PLATE 4

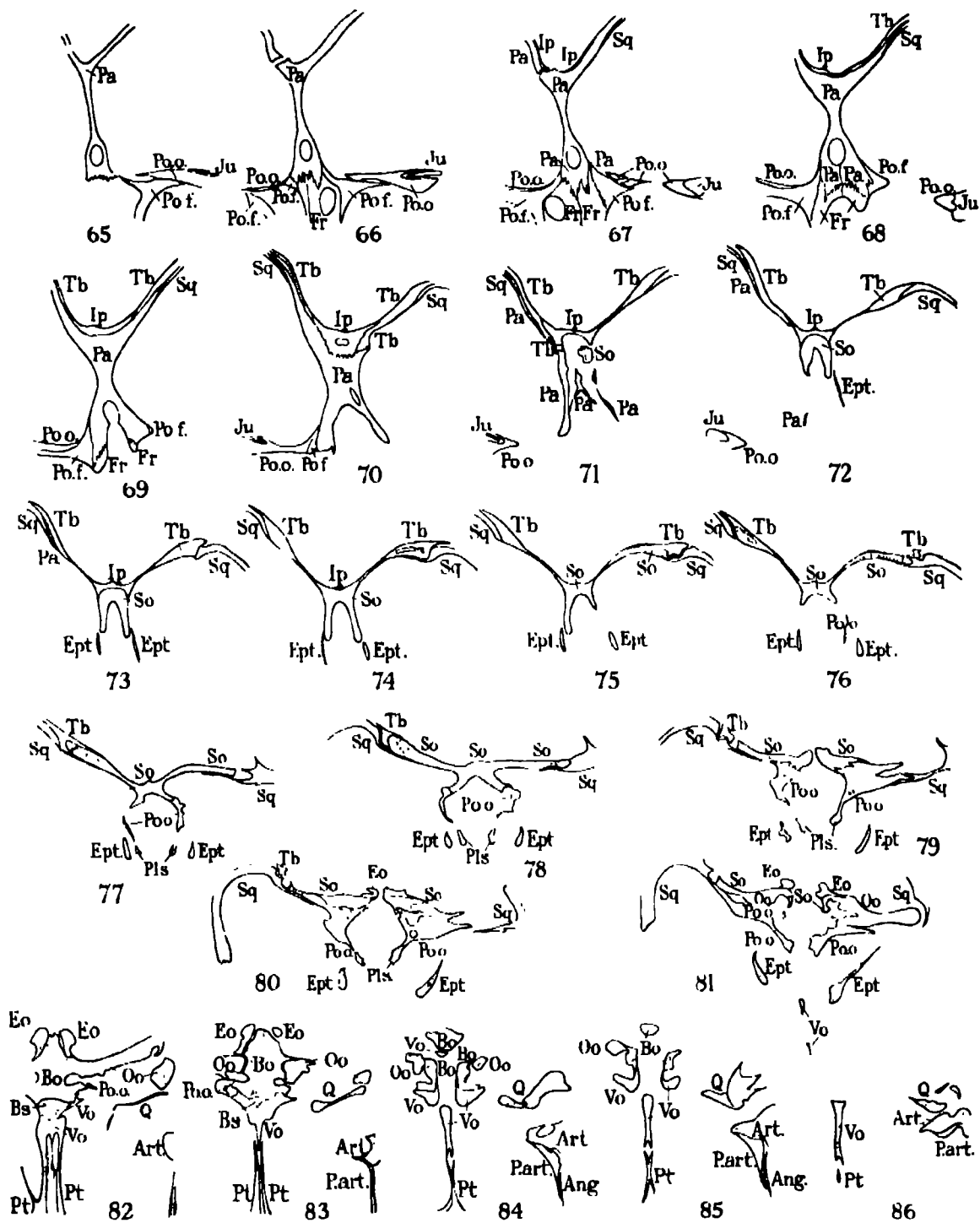
Figs. 52-64—Transverse sections of the posterior portion of the skull of probably *Trochosuchus acutus*,
Broom. $\frac{3}{8}$ natural size.



Trochosuchus acutus.

PLATE 5

**Figs. 65-86—Horizontal sections of the skull of a Therocephalian, most probably *Lycodops scholtzi*,
Broom. $\frac{2}{3}$ natural size.**

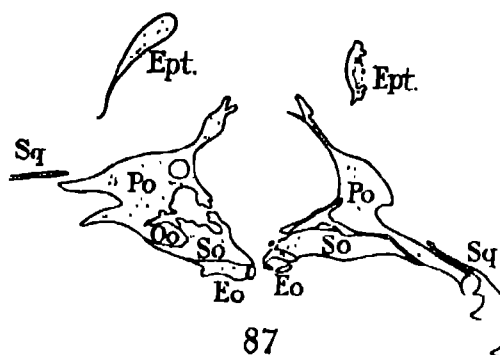


Probably *Lycedops scholtzi*.

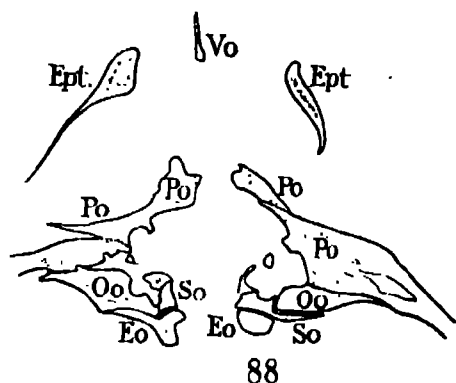
PLATE 6

Figs. 87-93—Horizontal sections of the brain-case, and the base of the skull of a Therocephalian, most probably *Lycedops scholtzi*, Broom. ♀ natural size.

Fig. 94—Restoration of the vomer of a Therocephalian, most probably *Lycedops scholtzi*, Broom ; as seen from below. ♀ natural size.



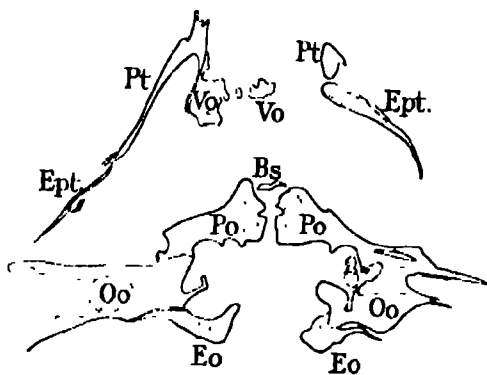
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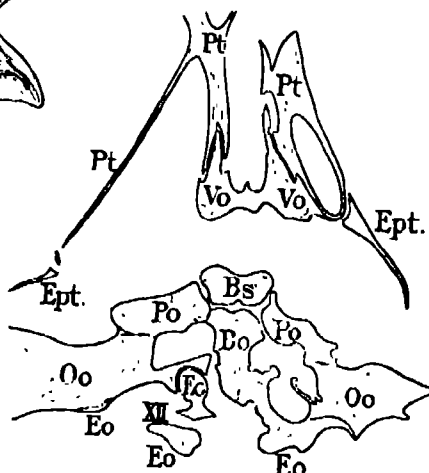
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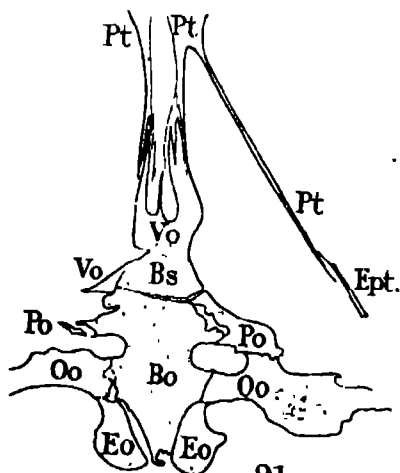
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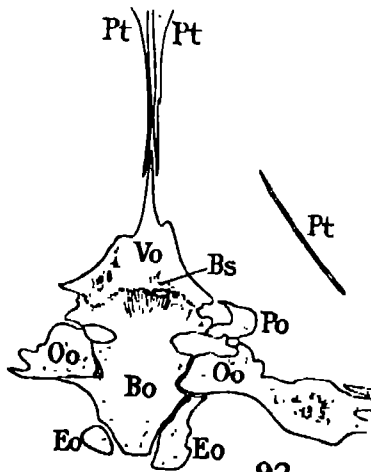
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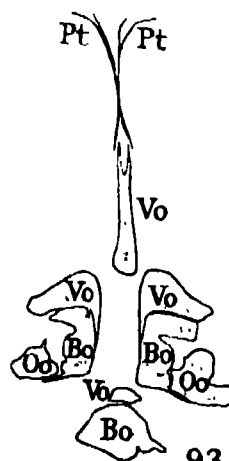
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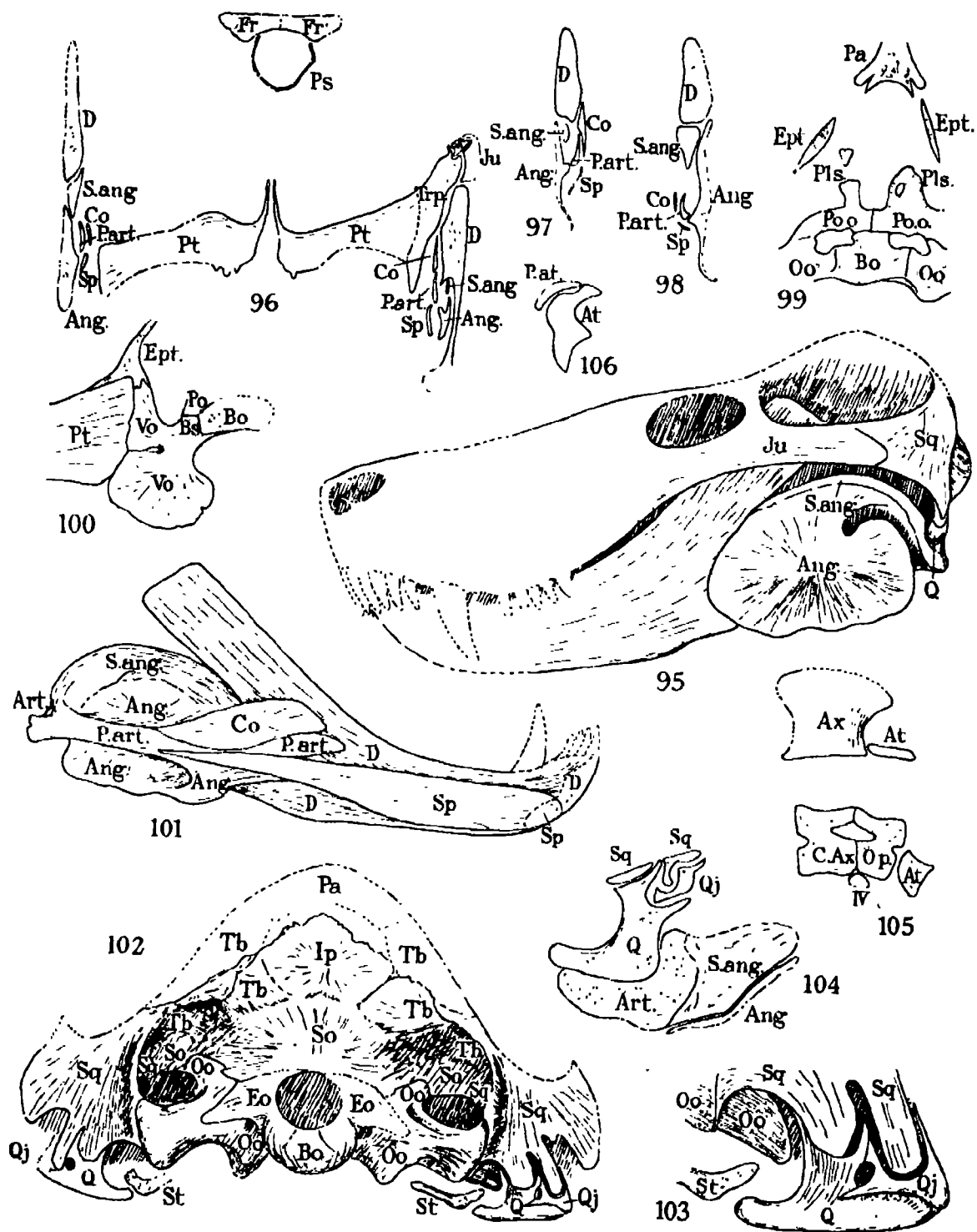


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Probably *Lycedops scholtzi*.

PLATE 7

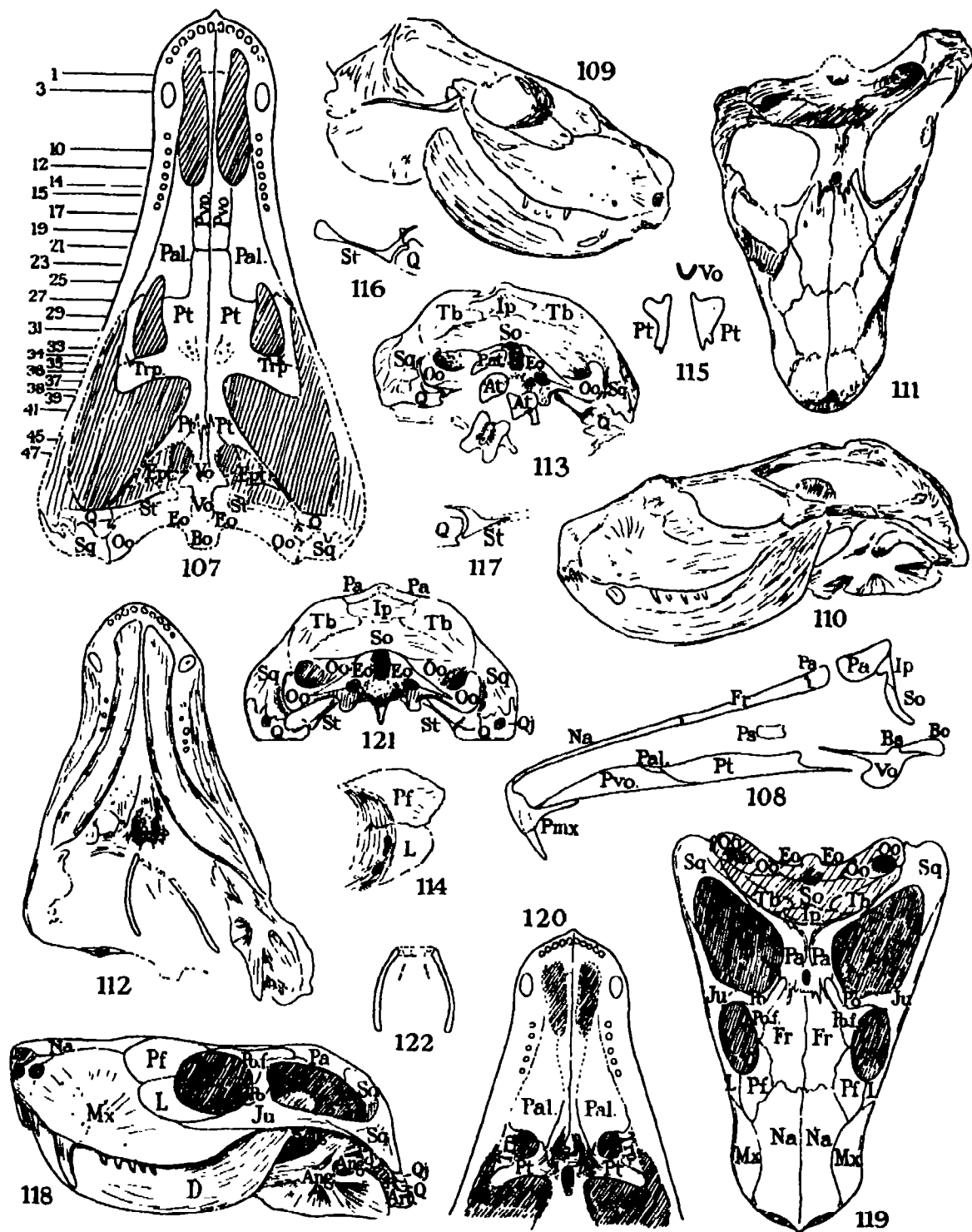
- Figs. 95-105—Various views and sections of the skull and upper vertebrae of *Lycedops scholtzi*, Broom.
- Fig. 95—Side view type skull of *Lycedops scholtzi*, Broom, partly restored. A portion of the snout in the canine region has been lost so that the exact length of the snout is uncertain. The posterior two-thirds of the skull are satisfactorily preserved. $\frac{1}{10}$ natural size.
- Fig. 96—A transverse section through the skull, showing the jaws in natural relations to the pterygoids, and the pre-sphenoid in natural relations to the frontals. $\frac{1}{10}$ natural size.
- Figs. 97-98—Section across the mandibles a little posterior to those shown in fig. 96. $\frac{1}{10}$ natural size.
- Fig. 99—A somewhat oblique transverse section through the back part of the skull, showing the basi-occipital, prootics, pleuro-sphenoids, and epipterygoids. $\frac{1}{10}$ natural size.
- Fig. 100—Side view of the vomer showing its relations to the basi-occipital, the basi-sphenoid, epipterygoid, and pterygoid. The basi-occipital, basi-sphenoid, prootic, and epipterygoid are shown in section. Almost the whole of the vomer and the part of the pterygoid shown are views of outer sides of these bones. $\frac{1}{10}$ natural size.
- Fig. 101—Inner view of left mandible—partly restored from sections. $\frac{1}{10}$ natural size.
- Fig. 102—Occiput showing most of the elements in undisturbed natural relations. $\frac{1}{10}$ natural size.
- Fig. 103—The right quadrate and related bones as seen from behind. 1 and $\frac{1}{10}$ natural size.
- Fig. 104—An oblique section through the right quadrate and related bones. 1 and $\frac{1}{10}$ natural size.
- Fig. 105—A section through the atlas and axis. $\frac{1}{10}$ natural size.
- Fig. 106—A section through the left atlantal arch and the left proatlas. $\frac{1}{10}$ natural size.



Lycedops scholtzi.

PLATE 8

- Fig. 107—Reconstruction of the palate of *Pristerognathus minor* (Haughton) from sections, and with the approximate position indicated of the sections figured in Plates 2 and 3. $\frac{2}{3}$ natural size.
- Fig. 108—A median diagrammatic section of the Therocephalian skull. The anterior two-thirds are reconstructed from the sections of *Pristerognathus minor*, and the posterior third reconstructed from the sections of the allied *Lycodops scholtzi*. $\frac{1}{10}$ natural size.
- Fig. 109—Right side view of the type skull of *Hofmeyria atavus*, Broom. $\frac{1}{8}$ natural size.
- Fig. 110—Left side view of the type skull of *Hofmeyria atavus*, Broom. $\frac{1}{8}$ natural size.
- Fig. 111—Top view of the skull of *Hofmeyria atavus*. As preserved. $\frac{1}{8}$ natural size.
- Fig. 112—Under view of skull of *Hofmeyria atavus*. As preserved. $\frac{1}{8}$ natural size.
- Fig. 113—Occiput with upper cervical vertebrae of *Hofmeyria atavus*, as preserved. $\frac{1}{8}$ natural size.
- Fig. 114—Oblique view of right pre-frontal and lacrimal bones of *Hofmeyria atavus*. $\frac{1}{8}$ natural size.
- Fig. 115—A section through the pterygoids and anterior part of the vomer of *Hofmeyria atavus*.
- Fig. 116—Right stapes and extra-stapedial of *Hofmeyria atavus*. 1 and $\frac{1}{8}$ natural size.
- Fig. 117—Part of left stapes and extra-stapedial of *Hofmeyria atavus*. 1 and $\frac{1}{8}$ natural size.
- Fig. 118—Side view of skull of *Hofmeyria atavus*, with the slight distortion corrected. $\frac{1}{8}$ natural size.
- Fig. 119—Top view of skull of *Hofmeyria atavus*, with the distortion corrected. $\frac{1}{8}$ natural size.
- Fig. 120—Restoration of the palate of *Hofmeyria atavus*. $\frac{1}{8}$ natural size.
- Fig. 121—Restoration of the occiput of *Hofmeyria atavus*. $\frac{1}{8}$ natural size.
- Fig. 122—Restoration of the hyo-branchial apparatus of *Hofmeyria atavus*. $\frac{1}{8}$ natural size.



Pristerognathus minor and *Hofmeyria atavus*.

PLATE 9

Figs. 123-132—Various views and sections of the skull, jaws, and parts of the skull of *Hyenosaurus platyceps*, Broom. Figs. 126 and 127 are $\frac{1}{2}$ natural size, all others are $\frac{1}{4}$ natural size.

Fig. 123—Upper view of the posterior third of the skull.

Fig. 124—View of the under side of the skull, partly restored in front.

Fig. 125—View of the occiput as preserved.

Fig. 126—Median section of vomer, showing the relations to the surrounding bones.

Fig. 127—Transverse section through the vomer, showing the vomer and its relations to the surrounding bones. The position of the base of the interorbital cartilage is indicated as a dotted structure.

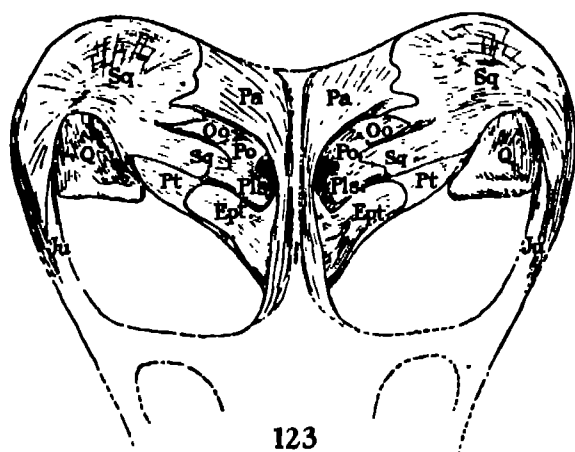
Fig. 128—Outer view of the left mandible, as preserved.

Fig. 129—Inner view of the right mandible, as preserved.

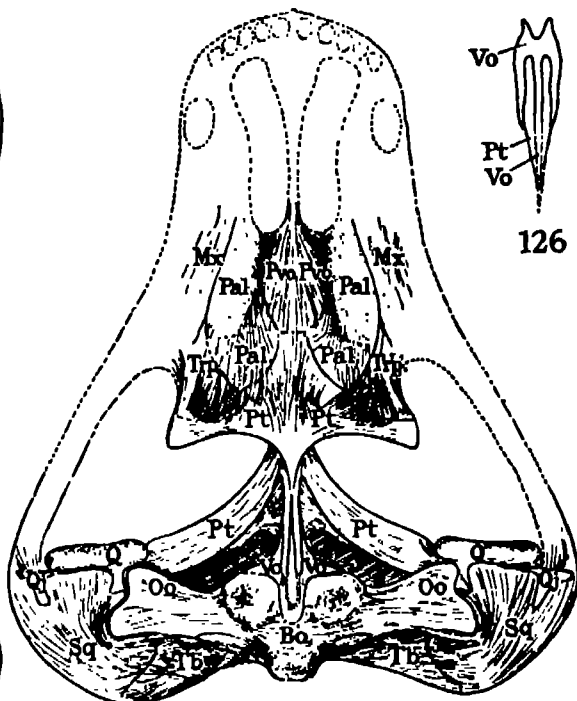
Fig. 130—Restoration of the occiput.

Fig. 131—Outer view of left mandible, restored.

Fig. 132—Inner view of right mandible, restored.



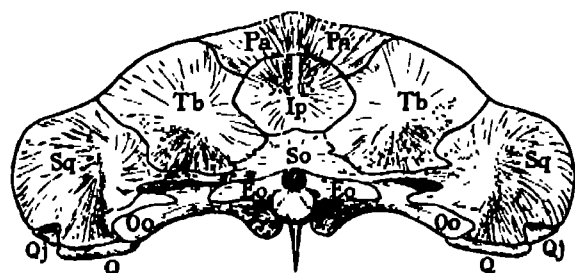
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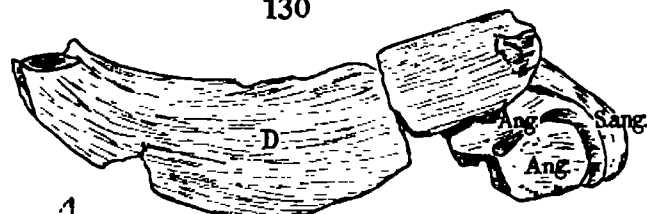
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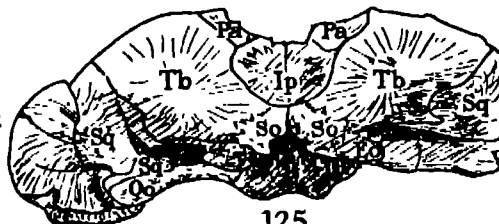
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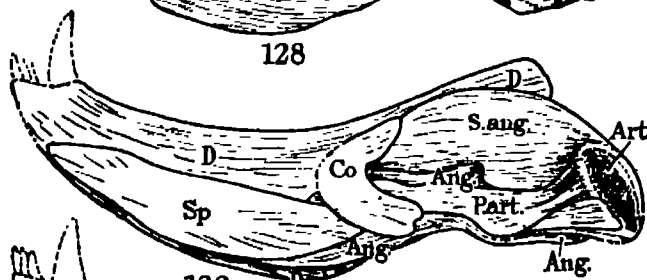
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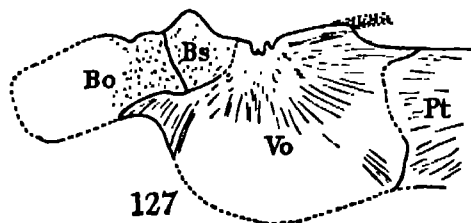
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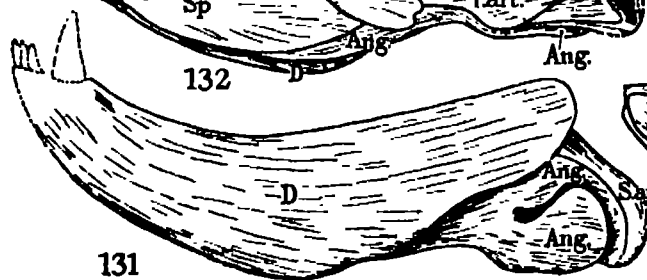
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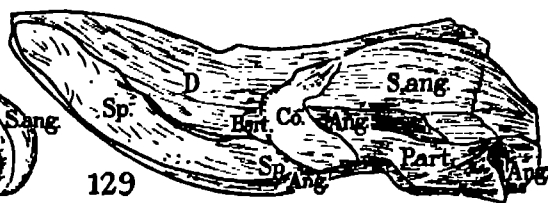
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II—Studies in Tunicate Development

Part V—The Evolution and Classification of Ascidians

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(Communicated by D. M. S. WATSON, F.R.S.—Received June 1—Read November 14, 1935)

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I—INTRODUCTION

The purpose of the present paper is twofold. The development and variability of the heart, pericardium, and epicardium throughout the group will be described, while an attempt is made to trace the probable course of evolution within the ascidians and to base upon it a revised classification as it concerns the major divisions of the class.

The mode of origin of the heart and pericardium has long been recognized as one of the strong links between the tunicates and vertebrates, while the epicardium is a highly significant structure assumed by many to be concerned primarily with budding. Current classifications of ascidians, such as those of LAHILLE and SEELIGER, are based upon the nature of the branchial sac, an insecure foundation since branchial structure is to a great extent an expression of the size of the organism; so that a more general basis is desirable.

A preliminary description is given of the adult structure and the development of *Ciona*,* inasmuch as there is good reason to believe that this genus represents

* References to original articles are not given for all species in order to save space, as most are well known.

closely the ancestral condition and there is no incontrovertible evidence of specialization.

II—MATERIAL

The material was studied in the form mostly of whole mounts and serial transverse sections. Frequently the circulatory system and other aspects of adult morphology were studied in the living animal.

Of the various genera described, *Ciona*, *Diazona*, *Ascidiella*, *Clavelina*, *Pycnoclavella*, *Archidistoma*, *Distaplia*, *Morchellium*, *Diplosoma*, *Botryllus*, *Polycarpa*, and *Molgula* were obtained while at the laboratory of the Marine Biological Association at Plymouth; *Perophora*, *Ecteinascidia*, and *Eudistoma* at the Bermuda Biological Station, to which institutions acknowledgments are made. *Euherdmania* was supplied by the Pacific Grove Biological Laboratory, *Rhopalea* by the Zoological Station at Naples; while *Tylobranchion* and *Colella* were examined through the courtesy of the British Museum (Natural History).

III—MORPHOLOGY AND DEVELOPMENT OF *Ciona*

Ciona is a comparatively large solitary ascidian. It is oviparous and without power to bud. Transverse blood vessels pass between the numerous rows of stigmata from the subendostylar vessel to the dorsal vessel. On the inner side of the branchial or pharyngeal wall longitudinal blood vessels occur, and where they cross the transverse vessels there is formed a bifid papilla projecting into the branchial cavity. Both LAHILLE (1890) and SEELIGER (1893-1907) have grouped *Ciona* with all such species bearing branchial papillae to form respectively the order Phlebobranchiata or Dictyobranchiata, comprising in reality a decidedly heterogeneous assortment.

The gut is coiled posteriorly to the branchial sac as shown in fig. 1, while the gonads lie in the loop of the gut and their ducts accompany the intestine to the atrial siphon. The retractile muscles of the siphons are formed by six muscle bands on each side, inserted posteriorly near the point of attachment of the individual to the substratum. The attachment itself is effected by numerous extensions of the test, this last material having been shown by BRIEN (1930) to be an external collagen secreted by mesenchyme cells that migrate through the epidermis.

The heart is a longitudinal invagination of the tubular pericardium, and opens at one end at the base of the endostyle into the sub-endostylar vessel, and at the other into vessels passing over the wall of the stomach. It is longer than the distance between these two regions and forms a V-shaped tube. Vessels in the test contain two channels separated by a mesenchymatous septum (*cf.* ÄRNBÄCK and BRIEN, 1932), blood flowing from one end of the heart to the terminal swelling of the vessel and returning to the other end.

The epicardium is in the form of a right and left perivisceral chamber opening by small apertures into the posterior end of the pharynx, fig. 1B. It is possible

that they have a general excretory function, for they allow water to bathe directly the pericardium, gonads, stomach, and intestine, no other excretory structures being known in *Ciona*.

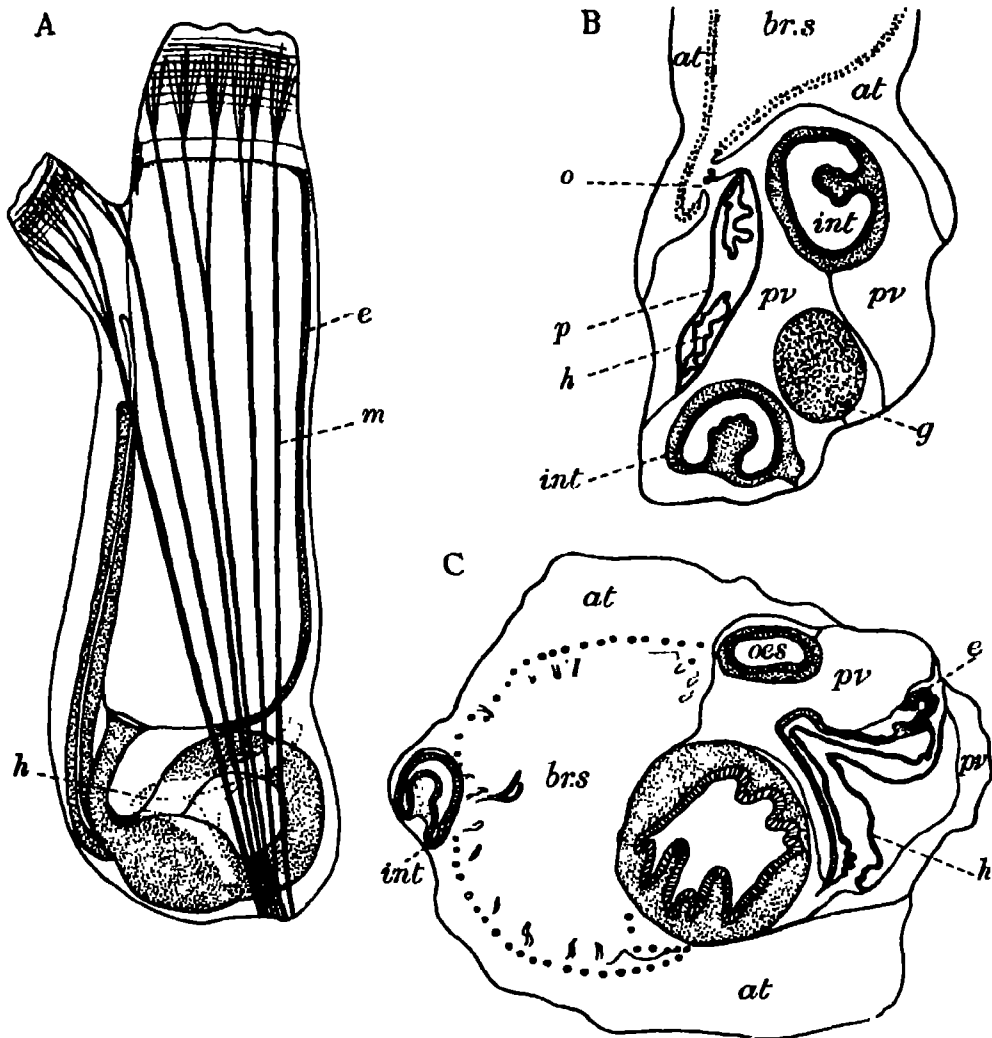


FIG. 1.—Morphology of *Ciona intestinalis*. A, adult from right side, showing muscle bands, digestive canal and heart; B, vertical section through base of adult showing heart and pericardium, digestive tube, gonad, and the openings of the perivisceral sacs (epicardia) into the pharynx; C, transverse section through small (5 mm) individual, showing various parts of digestive canal, and the heart with its openings to the stomach and endostyle. at, atrium; br. s, branchial sac; e, endostyle; g, gonad; h, heart; int, intestine; m, muscle; o, opening of epicardium to pharynx; oes, oesophagus; p, pericardium; pv, perivisceral sac.

In the course of development a tadpole larva is formed, and after its metamorphosis a small peculiar post-larval ascidian. The development and nature of the tadpole has little significance in the present connexion, for in its essentials there

is a remarkable uniformity throughout the ascidians, and tadpole variability, while of confirmatory value, is of secondary importance. The post-larval ascidian, however, differs in certain important respects from the adult. Correlated with its minute size there are but two protostigmata on each side in place of many rows of definitive stigmata. Also, in place of the single atrial siphon of the adult are two peribranchial siphons. These fuse eventually to form the atrial siphon at a time when the protostigmata have increased in number to six on each side and are becoming divided into a corresponding number of rows of stigmata. The pericardium develops as an evagination from the ventral wall of the pharynx, and the heart is formed by the infolding of the inner wall of the pericardium. At a slightly later stage when the protostigmata are functioning, the epicardia develop as two posterior evaginations from the pharynx to form the enveloping perivisceral cavities, with relatively wide openings into the pharynx, fig. 3B. At this stage the heart and pericardium is a short, straight tube passing from the base of the endostyle to the posterior end of the stomach, and only as the individual grows in size does the heart elongate. Since its two ends are fixed in position, elongation necessarily induces the V-shape characteristic of the adult, fig. 2.

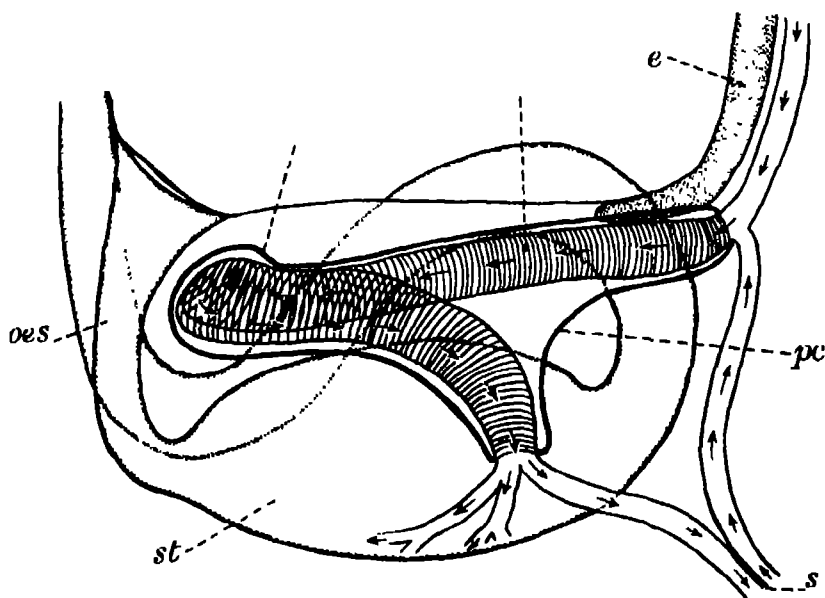


FIG. 2.—An enlarged view of the heart and pericardium of *Ciona* showing the relationship with the intestine and endostyle. *e*, endostyle; *pc*, pericardium; *oes*, oesophagus; *s*, septum; *st*, stomach.

In these early stages a structure is present that is absent in the adult. This is the post-abdomen, an epidermal stalk into which extend the retractile muscles of the siphons.

When the individual first functions as an ascidian the post-abdomen contains only mesenchyme in addition to the muscle fibres, but as growth proceeds a vessel

descends from each end of the heart, the two separated from one another by a mesenchymatous septum.

With further growth the post-abdomen shrinks and is represented in the adult only by the vessels of the test. In two rare varieties, however, of *Ciona intestinalis*, namely *v. longissima* and *v. gelatinosa*, according to ÄRNBÄCK and BRIEN (1932) the post-abdomen survives in the adult and may contain an extension of the left epicardium (perivisceral cavity) in addition to the muscle strands and blood vessels.

The above development is considered to be primitive. The adult structure to which it leads is also considered to be primitive, although there is the possibility that the stalk (post-abdomen) was once inhabited by the viscera as in many other forms. The V-shape of the heart and pericardium suggests this, but, as already described, that shape can readily be explained by the exigencies of growth. The power of budding may have been lost or may never have been acquired. If it once was present there is no reason to believe that it was of the type characteristic either of the perophorids or of the polystyelids, and the only likely method of budding in *Ciona* would be the abdominal constriction typical of the diazonids, distomids, and synoicids (*cf.* BERRILL, 1935, *b*). This could have occurred only if the gut-loop had descended into the stalk, and if the loop has always existed in the same relative position as it occurs in living *Ciona*, then abdominal budding is inconceivable. These two conclusions are therefore connected. If the position of the cionid gut is primitive, the absence of budding is also a primitive feature. If the position in the adult is secondary, then it is quite possible that abdominal budding once occurred. The position of the gut, however, is the same in the newly functional individual as it is in the adult, and the fact that this is the position typical of very young diazonids *before* the gut-loop descends into the stalk in those forms seems to confirm its primitive nature.

Thus no feature of the development or adult anatomy of *Ciona* can with certainty be said to be specialized, while there is some evidence that every such feature is a primitive character.

From this standpoint the remainder of the ascidians will be discussed.

From a cionid-like ancestor ascidians have apparently evolved in two main directions, one involving the descent of the viscera into the stalk, the other the shifting of the viscera forwards along the branchial wall. The first of these evolutionary trends gives rise to the order Aplousobranchiata (LAHILLE) or Kriko-branchiata (SEELIGER) together with the family Diazonidae. In fact, HARTMEYER (1923) saw such a close relationship between the diazonids and *Ciona* that he included them within one family.

IV—DESCENT OF THE VISCERA

Diazona, like *Ciona*, is an oviparous genus. It is the only genus with the faculty for budding that is so. It produces tadpoles like those of *Ciona*, while the post-larval ascidian can be distinguished from that of *Ciona* only by the relative larger

fixation stalk, a structure that grows from the anterior end of the tadpole. It is shown in fig. 3E, and there can be seen the two peribranchial siphons, the extensions of the muscle fibres to the base of the stalk, and the short tubular heart

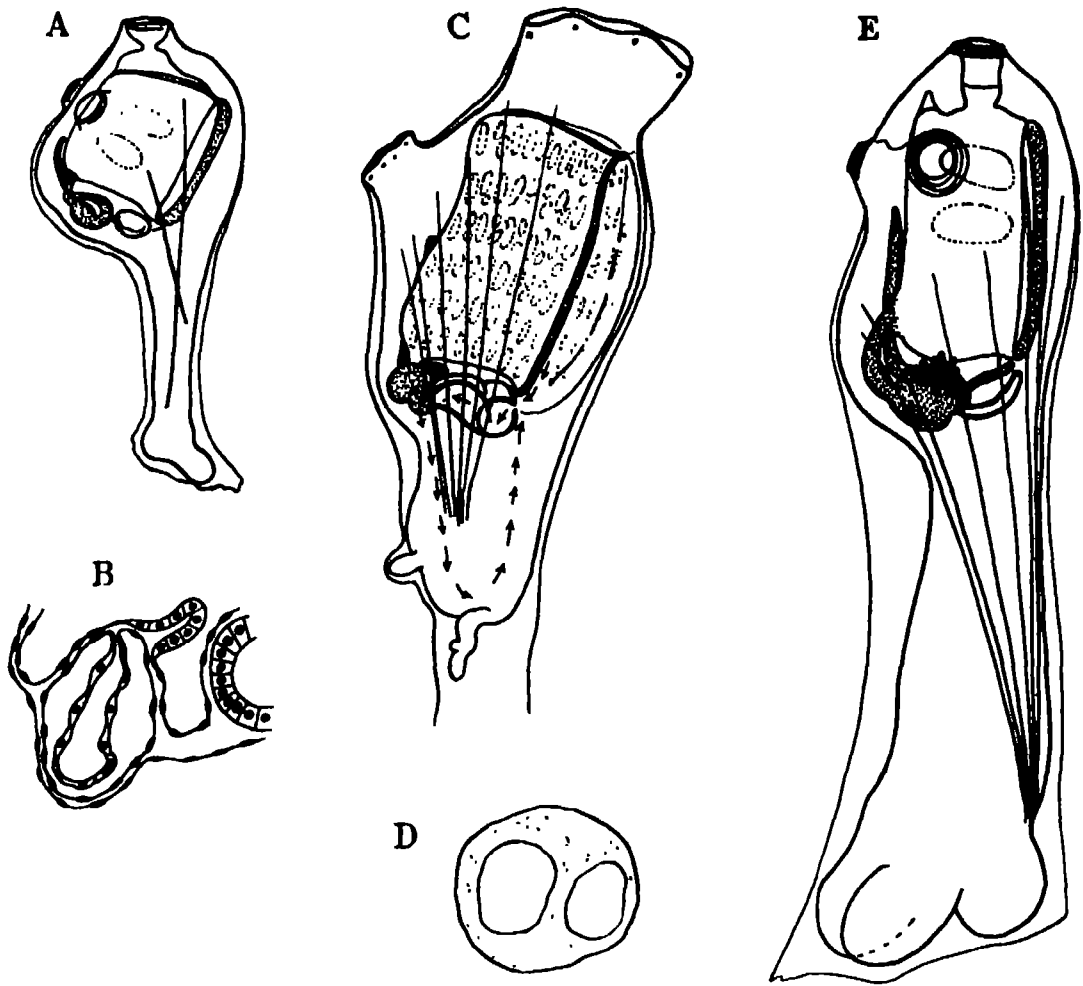


FIG. 3—The post-larval development of *Ciona* and *Diazona*. A, newly-functional *Ciona*, showing stalk with muscle fibres, the pair of peribranchial or atrial siphons, two protostigmata, and heart and gut-loop; B, vertical section of similar stage (after KUHN), showing the pair of perivisceral sacs or epicardia descending around the pericardium and heart; C, older *Ciona* with protostigmata divided into rows of definitive stigmata, single fused atrial siphon, circulation in stalk, and bending of heart; D, cross-section of stalk of stage C, showing blood sinuses and intervening mesenchymatous septum; E, newly-functional *Diazona*, similar to *Ciona* except for the hypertrophied stalk, showing non-descended gut-loop and simple straight heart. The illustrations are drawn to different scales, the stigmata in reality being approximately all of the same size.

extending from the posterior end of the stomach to the base of the endostyle. The gut is coiled beneath the branchial sac as in the young and adult of *Ciona*. At this stage the perivisceral sacs, again as in *Ciona*, are forming from the posterior end of the pharynx and have wide openings forward.

Nothing is known of the development between this stage and the adult condition, although there is no doubt that the peribranchial siphons fuse to form the median atrial siphon, and the protostigmata give rise to the rows of definitive stigmata,

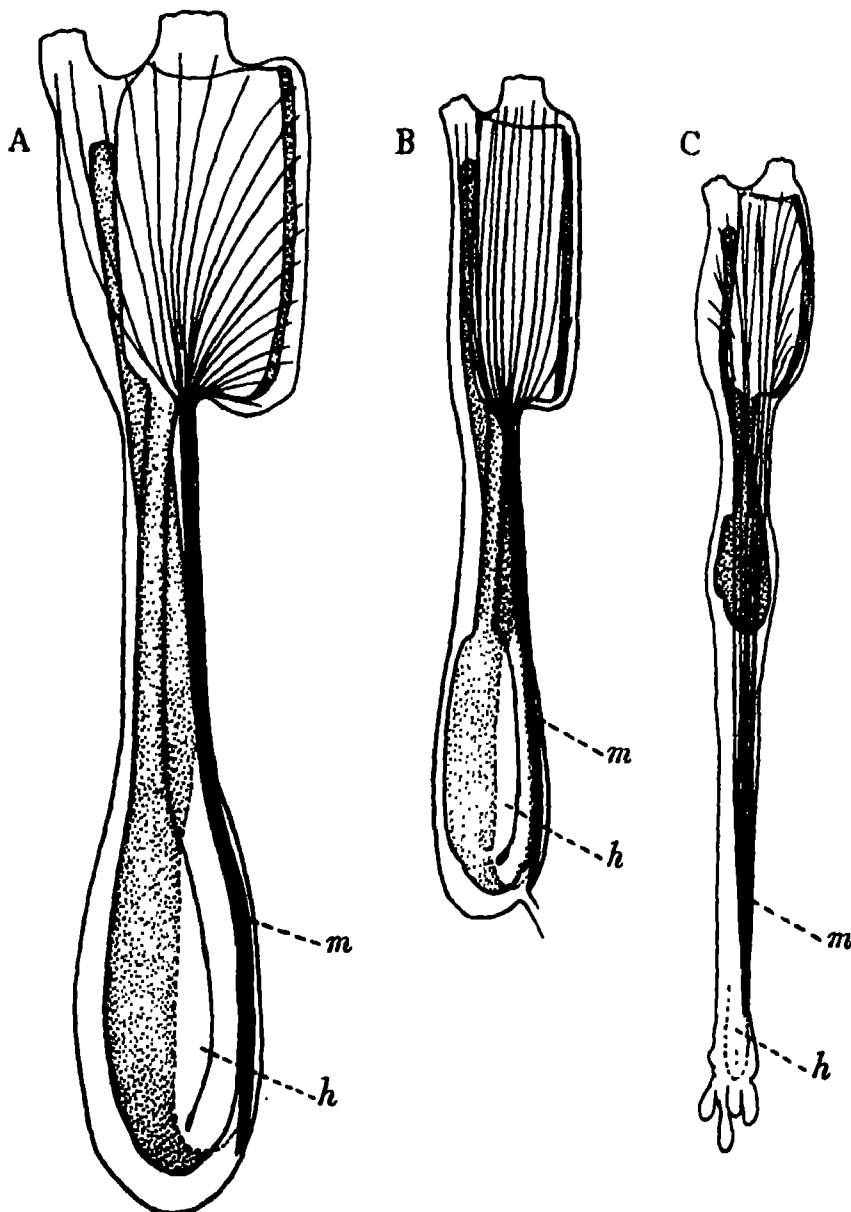


FIG. 4—A comparison of adult zooids of *Rhopalaea*, *Diazona*, and *Tylobranchion*, to show the musculature, the descent of the viscera into the stalk, and the relative position of the heart. In *Tylobranchion* the stalk is elongated, the posterior part forming the so-called "post-abdomen" containing heart, muscle, epicardia, and gonads. (Drawn approximately to the same scale): *h*, heart; *m*, muscle band.

as in *Ciona*. Internal longitudinal vessels are present in the adult, with papillae at their junctions with the transverse vessels. Apart from size the thorax of *Diazona*

and *Ciona* are remarkably alike, and this is the basis of their union within one family by HARTMEYER. In the abdominal region, however, the course of development must be markedly different, for in the adult the gut is no longer coiled beneath the branchial sac as in all stages of *Ciona* and in the post-larval stage of *Diazona*, but has descended to the base of the stalk, carrying with it the heart, gonads and perivisceral sacs. The test vessel persists as in *Ciona*, and is composed of a blood sinus from each end of the heart separated by a mesenchymatous septum that seems to take origin from the pericardium. These relationships are to be seen in fig. 5E. The two ends of the heart are still related to the stomach and the base of the endostyle, but the heart itself is more definitely V-shaped with the acute angle

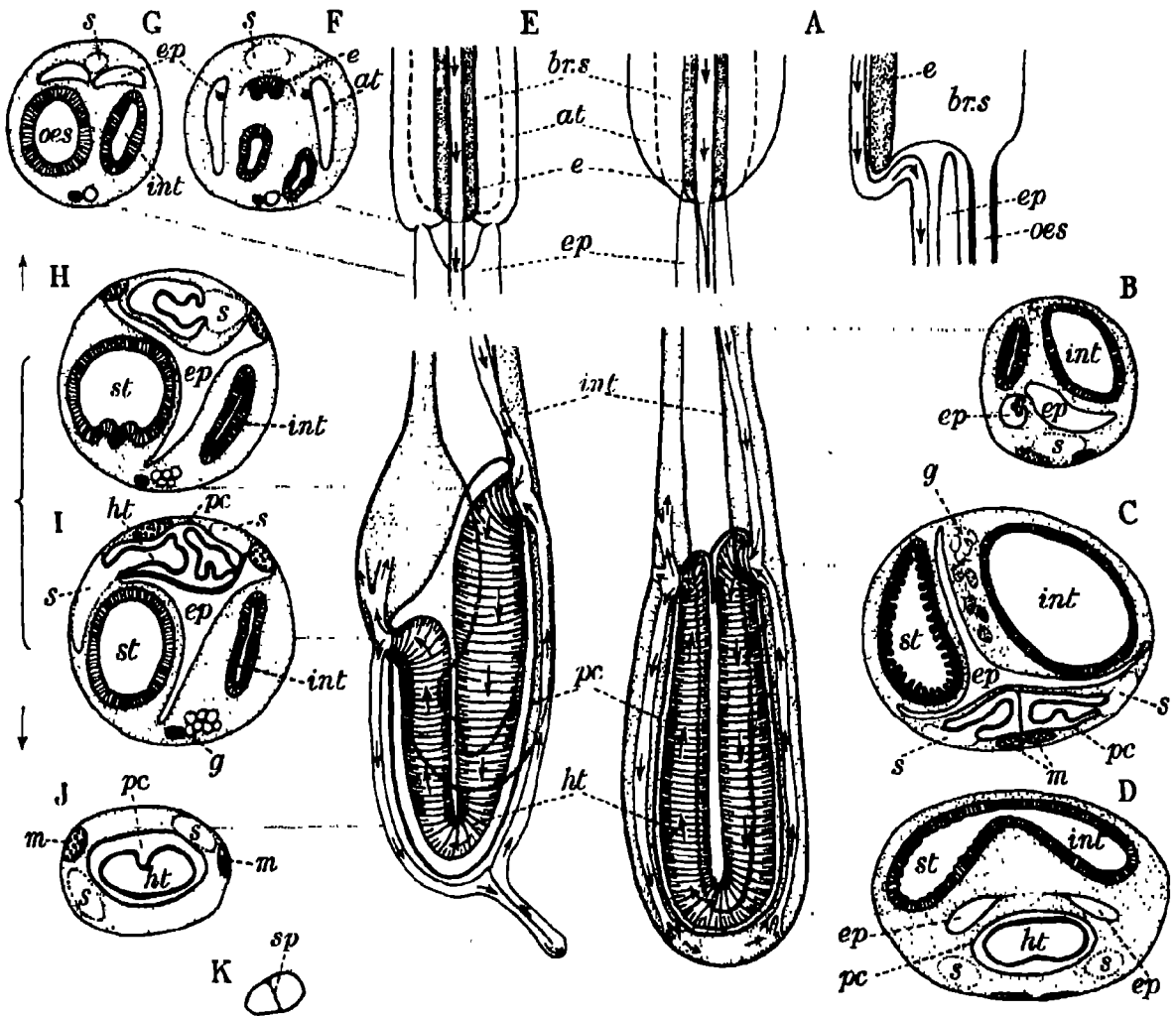


FIG. 5.—Transverse sections and reconstructions of the post-branchial region of *Rhopalea* and of *Diazona*. A, reconstruction; B, C, D, sections of *Rhopalea*; E, reconstruction; F–K, sections of *Diazona*. at, atrial cavity; br.s, branchial sac; e, endostyle; ep, epicardium; g, gonad; ht, heart; int, intestine; m, muscle band; oes, oesophagus; pc, pericardium; s, blood sinus; sp, mesenchymatous septum; st, stomach.

forming at the base of the stalk. A further change is to be found in the nature of the perivisceral sacs. These descend with the rest of the viscera and lie between the gut-loop and the heart, but instead of remaining separate from one another and with openings into the branchial sac or pharynx, the two sacs fuse to form a single chamber through the greater part of their length. The original openings close, although one of the anterior horns gains a secondary opening not into the pharynx, but into the peribranchial cavity, fig. 5F. This again suggests an excretory function of the perivisceral cavity (or epicardium), especially as secretions into the lumen are noticeable in *Diazona* and many other forms.

Diazona is a colonial form, and buds by a process of abdominal constriction, the constrictions being an epidermal phenomenon, while the cells responsible for the reorganization and regeneration come from the wall of the epicardium (BERRILL, 1935, *b*).

Rhopalea in some ways is a link between *Diazona* and *Ciona*. It is usually solitary and intermediate in size between the two forms, the power to bud being suspected but not proved to exist. Its general structure is shown in figs. 4 and 5. It differs from *Diazona* only in that the two limbs of the heart are of approximately equal length, there is no ventral test vessel, and there is no secondary opening of the epicardium into the peribranchial cavity.

V—INFLUENCE OF DWARFING

The cionid-diazonid stock undoubtedly represents the primitive ascidian type, and, as already suggested, there is evidence that the cionid condition is more primitive than the diazonid, rather than derived from it.

The remaining diazonid genus is *Tylobranchion*, a rare form of the South Atlantic. The zooids and colonies are smaller than those of *Diazona*. This question of absolute size has already been discussed (BERRILL, 1935, *a, b*), but it may be noted here that reduction in size of a colony reduces the volume more than the surface, so that reduction tends to cause congestion of the posterior ends of the zooids embedded in the mass of test. In order to overcome such posterior crowding, two modifications appear. One is the extreme shortening of the zooid, the other is its relative elongation so that it becomes proportionately slender. Thus a zooid of *Tylobranchion* is much more cone-shaped than is one of *Diazona*, and this has been brought about by a growth of the stalk between its base and the posterior end of the abdomen. Into this post-abdominal stalk extend the muscle fibres, the gonads, the epicardium, and the heart, so that the zooid as a whole becomes relatively longer and narrower. Rather than a growth of the stalk, into which certain organs extend, it should be imagined that the whole stalk and contents extend posteriorly with the exception of the intestinal loop.

In all species of *Tylobranchion* the transverse vessels bear bifid papillae, but in none do they unite to form longitudinal vessels (ÄRNBÄCK, 1926). This may be

correlated with the small size of the zooids, for in *Rhopalea norvegica* (ÄRNBÄCK, 1925) the papillae are united to form longitudinal vessels in an individual of 20 mm length, but in one of 14 mm there are merely transverse vessels bearing bifid papillae. In other words, only when individuals exceed a certain size do the papillae unite to form longitudinal vessels. In *Rhopalea* this critical size lies between 14 and 20 mm length, and since the average size of the three known species of *Tylobranchion* is about 5, 11, and 14 mm respectively, the absence of longitudinal vessels is understandable. This subject has been emphasized since the presence of longitudinal vessels and the secondary papillae born by them has been used to define the order Ptychobranchiata (Phlebobranchiata). If dwarfing involves their loss or non-development, then obviously they have limited diagnostic value.

Among the Krikobranchia (Aplousobranchia) there are many types; were it not for the absence of inner longitudinal vessels in the branchial wall they would be recognized as being closely related to the diazonids. Thus the distomids, *Archidistoma* and *Eudistoma*, can be regarded as dwarfed diazonids. The stalk contains the gut-loop, gonads, heart and epicardium, as in *Diazona*. The epicardia are fused and have lost the openings into the pharynx. There is a narrow vessel to the test, containing a mesenchymatous septum; and budding is by abdominal constriction (BERRILL, 1935, *b*). The zooids and colonies are very small and are, in fact, dwarfed diazonid types, there being associated with dwarfness a loss of the internal longitudinal vessels and the papillae that give rise to them, and as in most dwarfed ascidians, the eggs are few and large, giving rise to comparatively elaborate tadpole larvae.

Reconstructions of the abdominal region of *Archidistoma*, *Eudistoma*, and *Pycnoclavella* are to be seen in fig. 6, showing the relative positions of gut-loop and epicardium, and the U-shaped heart, which in these very small zooids is comparatively short.

Closely related to the above distomids are *Distaplia* and *Colella*, two forms that have further elaborated the colonial state. The zooids are again very small and short, but become arranged in the colony in definite systems. They are also specialized in their method of budding (*cf.* BERRILL, 1935, *b*). Structurally they differ from *Archidistoma* or *Eudistoma* in that the ventral test vessel is enormously hypertrophied and contains wide blood channels, separated as in previously mentioned genera by a mesenchymatous septum arising from or near the pericardial wall. The vessel has a twofold significance. In *Distaplia* it is sterile, buds arising in the oesophageal region, but in *Colella* the stolon constricts into buds and the septum is the source of the totipotent cells. There is also an influence on the heart. The enlargement of the stolon blood vessel has resulted in a reduction of the shorter limb of the heart, so that it opens at one end at the base of the stalk where the stolon blood vessel takes origin, fig. 7.

Clavelina, fig. 8, is similar to the distomids in having a hypertrophied stolon blood vessel and a heart that has been shortened and straightened to open at one end into the stolon blood vessel and at the other towards the endostyle. As in *Colella*,

the buds arise from the fragmented stolon with the totipotent cells supplied by the septum.

Clavelina and such closely related genera as *Podoclavella* and *Chondrostachys*, alone among the Krikobranchia, might be expected on the basis of size of zooid to possess branchial papillae and internal longitudinal vessels. Since, however, there are indications that the relatively large size is a secondary development, and that these forms may have evolved from the smaller distomids, the absence of such structures may not have much significance. The loss associated with dwarfness might readily be permanent. As in the distomids, the septum of the stolon vessel

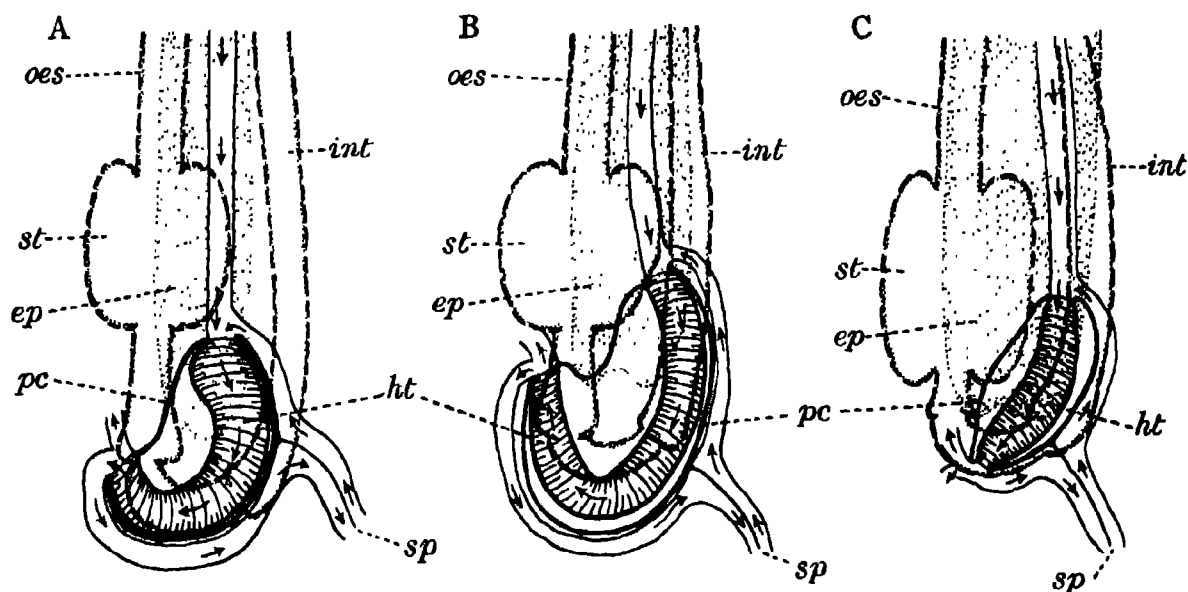


FIG. 6—Reconstructions of the abdominal region based upon whole mounts and transverse sections of A, *Archidistoma aggregata*; B, *Eudistoma olivacea*; and C, *Pycnoclavella aurilucens*. *ep*, epicardium; *ht*, heart; *int*, intestine; *oes*, oesophagus; *pc*, pericardium; *sp*, mesenchymatous septum of vascular stolon; *st*, stomach.

is mesenchymatous and arises at the base of the pericardium. This is so in both blastozooid and oozooid of *Clavelina*, and nowhere does it arise from the epicardium as stated, though not figured, by VAN BENEDEN and JULIN (1886) (*cf.* BERRILL, 1935, *b*). There is, in fact, no evidence at all that the stolon vessel is in any way associated with the epicardium, and the epicardium cannot be said to be an organ primarily concerned with budding, except in certain highly specialized forms.

One of such forms is *Diplosoma* (and the family to which it belongs, the Didemnidae). The gut-loop and heart is similar to that of *Distaplia* or *Colella*, although the ventral stolon vessel has disappeared.

In this genus the epicardia or perivisceral sacs are so small that they of necessity remain separate from one another, and they undoubtedly function primarily as

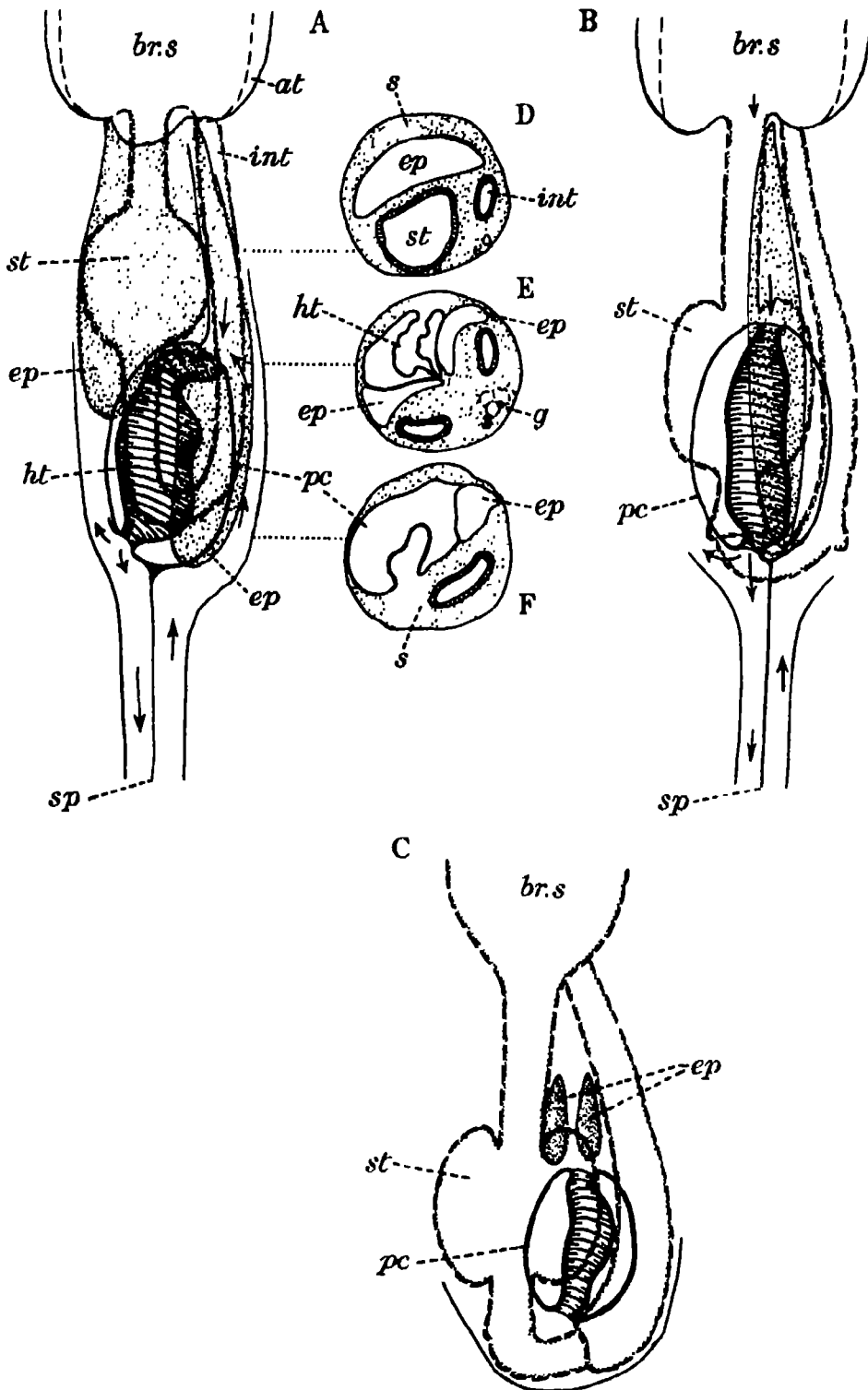


FIG. 7.—Reconstructions of A, *Colella thomsoni*; B, *Distaplia rosea*; C, *Diplosoma gelatinosa*; D, E, F, transverse sections of *Colella*. *at*, atrial cavity; *br. s*, branchial sac; *ep*, epicardia; *g*, gonad; *ht*, heart; *int*, intestine; *pc*, pericardium; *s*, blood sinus; *sp*, stolonic septum; *st*, stomach.

organs that give rise to the totipotent cell masses during the complex budding process. Budding is of a type related to that of *Distaplia* (BERRILL, 1935, b). The disposition of the various organs is shown in fig. 7C.

In the other forms just mentioned, namely, *Archidistoma*, *Eudistoma*, *Pycnoclavella*, *Distaplia*, *Colella*, and *Clavelina*, the epicardium is an extensive chamber resulting

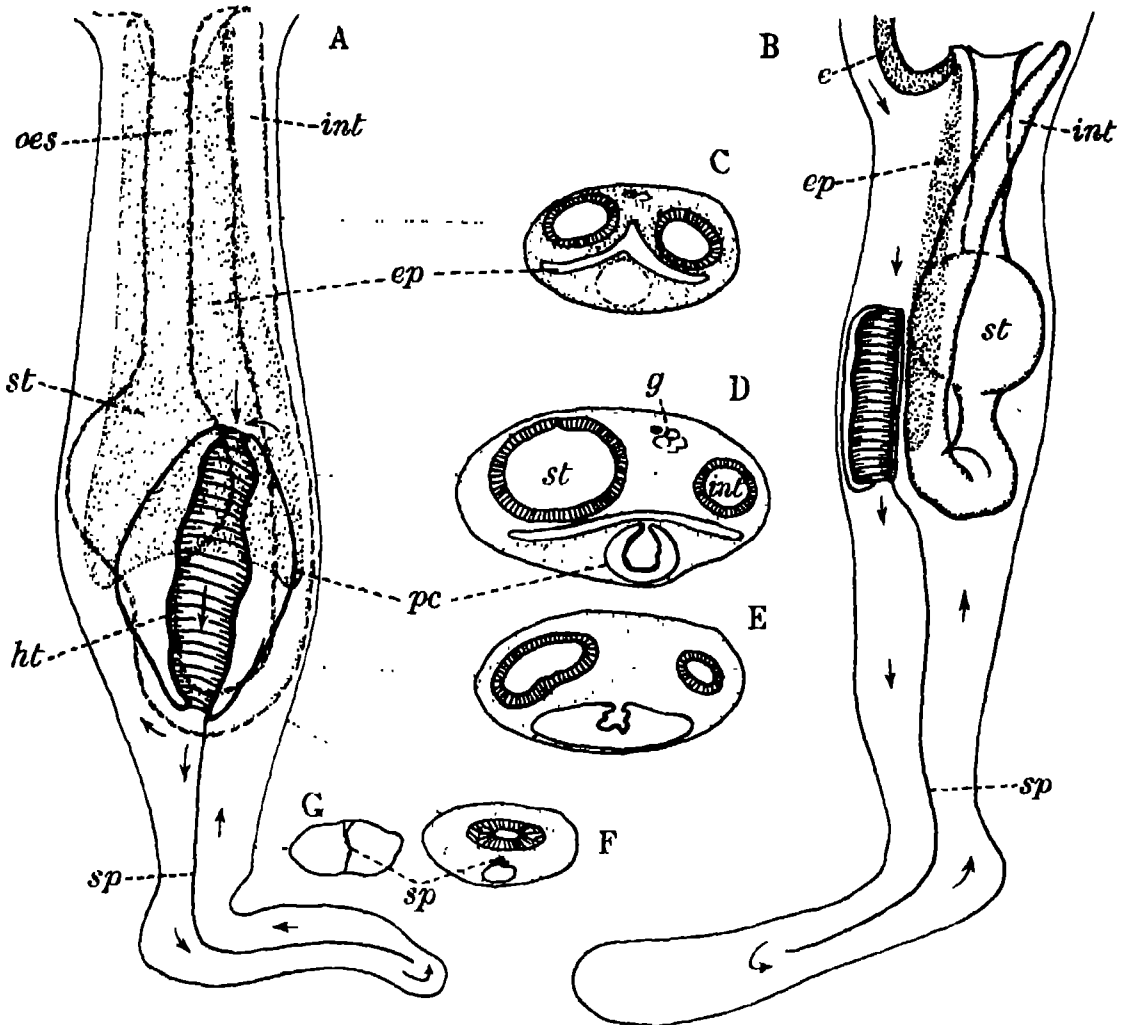


FIG. 8—*Clavelina lepadiformis*. A, reconstruction of adult blastozoid, from ventral side; B, whole mount drawing of young oozoid from side showing relation of stolon to pericardium; C-G, transverse sections of adult blastozoid. *e*, endostyle; *ep*, epicardium; *g*, gonad; *ht*, heart; *int*, intestine; *oes*, oesophagus; *pc*, pericardium; *sp*, stolon; *st*, stomach.

from the fusion of the right and left perivisceral sacs. During development these develop, as in *Ciona*, from posterior diverticula of the pharynx, but in the adults the pharyngeal connexions are lost and the paired condition is denoted only by the pair of anterior horns. The chamber lies always between the gut-loop and the heart, as is indicated by the transverse sections shown in figs. 7 and 8.

Originating from a diazonid stock, zooids have responded to a dwarfing of the colony not only by a shortening of the body as a whole, but also by a narrowing and extension of the posterior parts, much as in *Tylobranchion*. This has occurred in the family Synoicidae. The stalk elongates, the muscle insertions, the posterior end of the epicardium, and the bend of the heart retain their position relative to the base of the stalk and give the appearance of having descended into the extended stalk, since the gut-loop does not lengthen in a corresponding manner. On the side of the epicardium opposite the heart the gonads are to be found also in the post-abdominal stalk. As there is no posterior formation of a stolon-like vessel, there has been no tendency for the U-shaped heart to become straightened out as in the distomids and *Clavelina*.

One genus remains that needs special mention. *Euherdmania* has zooids elongated as in the Synoicidae, but with two important differences: the gut-loop has descended into the extended stalk to a greater distance, while the two epicardial sacs, though losing their openings into the pharynx, remain distinct from one another throughout their length.

The above types are illustrated in fig. 9.

Thus, in one direction the cionid type has evolved through the extension and occupation by the viscera of the fixation stalk. While associated with a reduction in size of colonies among such forms, there has been a dwarfing of the zooid as a whole and in some a hypertrophy of the ventral stolon-like vessel, on the one hand, and an extension and narrowing of the body to form a post-abdomen containing all the viscera but the gut-loop on the other.

VI—ASCENT OF THE VISCERA

Other ascidians, however, have evolved from the cionid type in a very different direction. In most of these there has been an elaboration of the branchial sac, associated in all probability with increase in absolute body size, since such increase demands a relatively greater increase of respiratory and feeding surfaces. The branchial sac tends to extend posteriorly between the gut-loop and the heart, and in adult forms these two structures become separated from one another and lie on opposite sides of the pharynx. There is, in fact, not only an extension of the branchial sac posteriorly, but also a dislocation or migration forwards of the gut-loop and associated gonads, and the heart and pericardium.

With increase in individual body size and hypertrophy of the branchial sac the fixation stalk dwindles even to a greater extent than it does in *Ciona*. In the Ascidiidae short sub-terminal ampullae apparently represent the stalk shortly after metamorphosis, but not for long. In the families of the Stolidobranchia (Ptychobranchia) the stalk again is recognizable in some species during or shortly after metamorphosis, but either it is without obvious function as in *Botryllus*, *Symplegma*, or *Distomus*, or it forms a creeping stolon-like structure as in certain Molgulae and in *Styela* and *Polycarpa* (BERRILL, 1929, 1931). In many species (*Botryllus*, *Symplegma*,

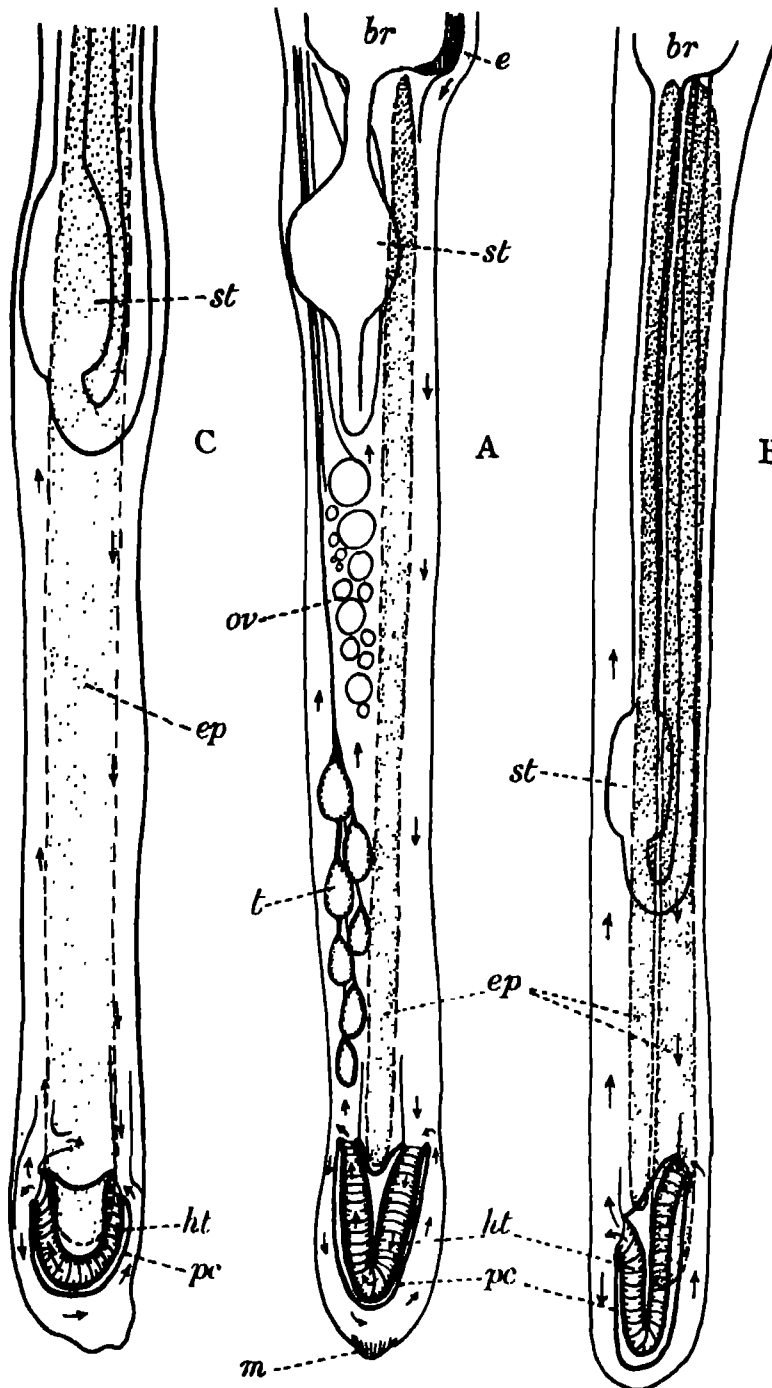


FIG. 9—Reconstructions of abdominal and post-abdominal regions of A, *Sidnyum*; B, *Fuherdmania*; and C, *Tylobranchion*, based upon whole mounts and transverse sections. *br*, branchial sac; *e*, endostyle; *ep*, epicardium; *ht*, heart; *m*, posterior insertion of muscle bands; *ov*, ovary; *pc*, pericardium; *st*, stomach; *t*, testes.

Distomus, *Styelopsis*, etc.) the metamorphosing zooid becomes attached by a ring of ectodermal ampullae surrounding the rudiment of the stalk, and the zooid is compressed against the substratum.

In both groups the Ascidiidae and the Stolidobranchia, the stomach and intestinal loop, with its contained gonads, becomes shifted forwards to lie along one side of the branchial sac, while the heart tends to shift forwards along the other side. In spite of such dislocation one end of the heart still opens into the subendostylar vessel, though no longer at the base of the endostyle, and the other end in the region of the stomach.

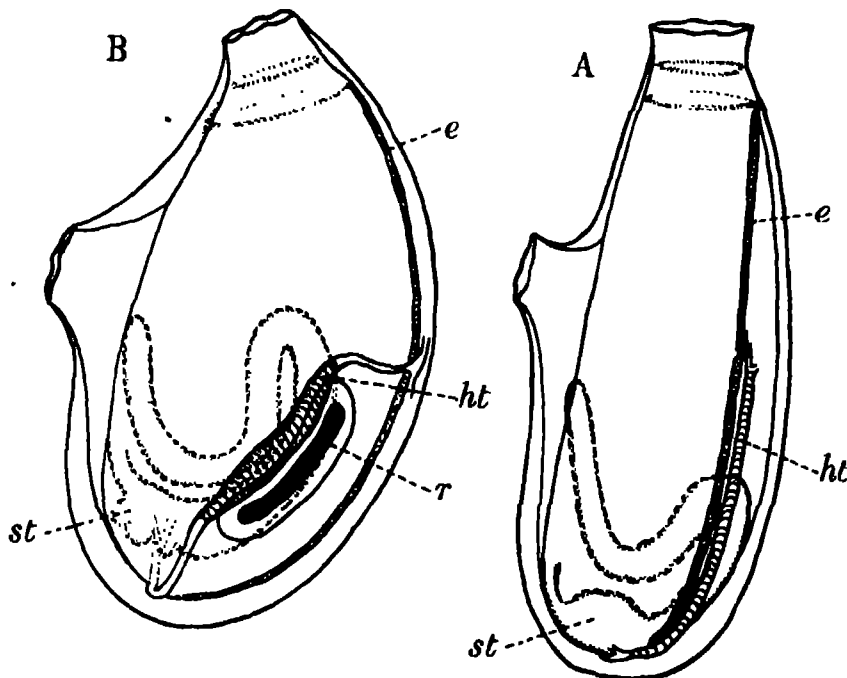


FIG. 10.—Adult morphology of *Ascidia* (A) and *Molgula* (B), showing hypertrophy of branchial sac, and translocation of heart and digestive tube, in *Molgula* to opposite sides of sac. *e*, endostyle; *ht*, heart; *r*, renal vesicle; *st*, stomach.

With the shifting of the viscera forwards to the right and left, the perivisceral sacs or epicardia, as they exist in *Giona* or in *Diazona*, can no longer retain their original relationships. As cavities allowing water to flow over the gut, heart and gonads, their function has to a considerable extent been taken over by the peribranchial cavity or atrial chamber. They do not, however, disappear entirely.

In 1902 DAMAS described the development of the heart in *Molgula* and discovered that during metamorphosis two vesicles are formed from the base of the pharynx. They are in close contact one with another, the one developing to form the heart and pericardium, the other to form the renal vesicle with its enclosed precipitate. The renal vesicle remains in contact with the heart throughout the life of the individual, the two organs growing and elongating together. There is every reason

to believe that the renal vesicle is homologous with the epicardium. It is formed from the same region of the pharynx at the same stage of development as the epicardial sacs in *Ciona*. Moreover, the renal function of the epicardia is indicated on other grounds, in the appearance of secretions in those of various forms, and in the establishment of secondary openings into the atrial cavity in *Diazona*. Even in the adult *Molgula* the renal vesicle bears the same relationship to the heart as the epicardium does to that organ in such forms as *Clavelina*, or *Distaplia*.

The renal vesicle remains a single chamber in *Molgula*, and it can be seen in the recently metamorphosed forms of any species of that genus. It can also be seen in the corresponding stage in at least two other genera. As shown in fig. 11, it is discernible also in very young individuals of *Polycarpa* of the family Styelidae, and of *Ascidiella* of the family Ascidiidae, and in *Ascidiella* at least it develops as in *Molgula*, from the base of the pharynx.

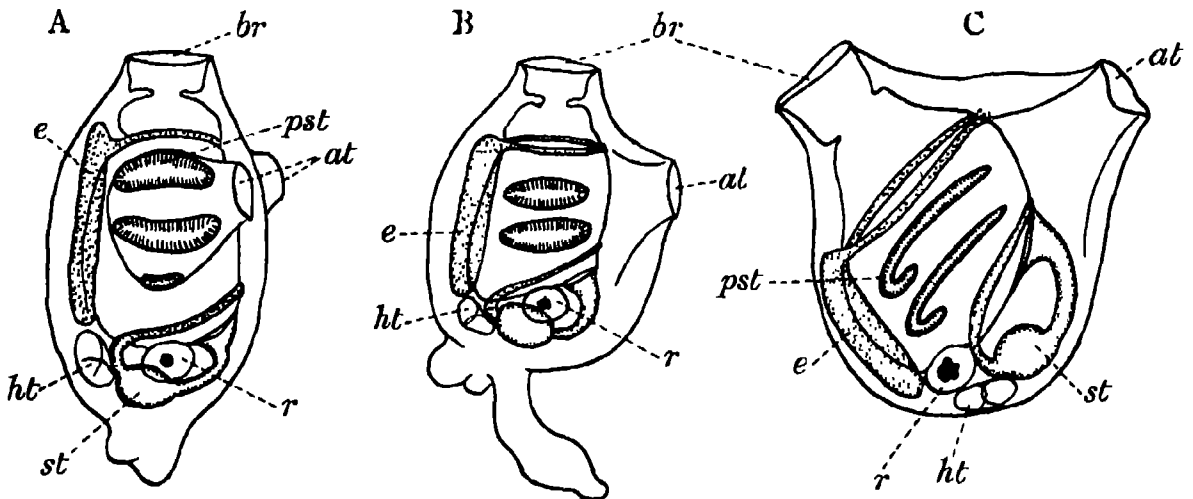


FIG. 11—Newly-functional oozoids of A, *Ascidiella aspersa*; B, *Polycarpa rustica*; and C, *Molgula citrina*. at, atrial siphon (s); br, branchial siphon; e, endostyle; ht, heart; pst, protostigma; r, renal vesicle (epicardium); st, stomach.

Thus the Ascidiidae and Stolidobranchia are united together by the dislocation of the viscera and the transformation of the perivisceral sacs or epicardia into a renal vesicle. In this last respect there is more uniformity among the recently metamorphosed individuals than among their respective adults. Only in *Molgula* does the renal vesicle remain single and retain its primitive relationship to the heart. In the Ascidiidae and *Corella* the vesicle, instead of growing as a single organ, multiplies or subdivides until the gut wall and region between gut and heart becomes congested with innumerable small renal vesicles.

In the Styelidae, Botryllidae and some Pyuridae, the vesicle or vesicles are not recognizable in the adult, although it is discernible in *Microcosmus*, a genus linking the Pyuridae with the Molgulidae.

In other respects the Stolidobranchia and the Ascidiidae differ considerably. The Ascidiidae are the more primitive in that they form a pair of lateral peribranchial chambers in development that later fuse to form the median atrium. In the Stolidobranchia the atrial aperture is median and single from the very beginning. The gonads in the Ascidiidae are to be found only within the loop of the gut, while in the Stolidobranchia bilaterality is more pronounced and gonads are formed on the opposite side as well.

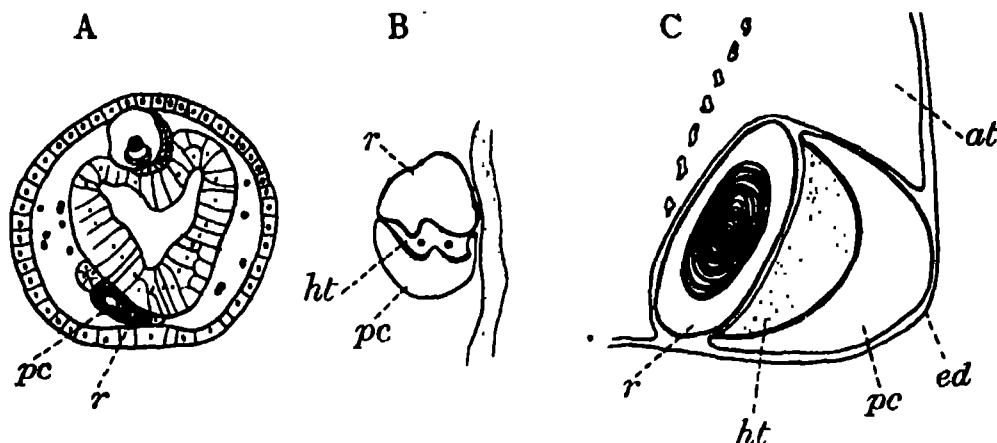


FIG. 12—Relationship between renal vesicle and heart in *Molgula*. A, origin of pericardium and renal vesicle from base of pharynx in tadpole of *Molgula echinosiphonica* (after DAMAS); B, renal vesicle, heart, and pericardium in *Molgula* similar to stage shown in fig. 11 C (after DAMAS); C, condition in adult *Molgula*. at, atrial cavity; ed, epidermis; ht, heart; pc, pericardium; r, renal vesicle.

The evolution from a cionid type to form the above-mentioned groups may thus be visualized as occurring in two stages. In the first, as represented by the Ascidiidae, there is a translocation of the gut-loop and gonads along one side and the heart towards the other side of the branchial sac, while the epicardia are transformed into the renal vesicle. In the second there is intensification of bilaterality as evidenced by gonads on each side, a developmental abbreviation in the formation of the atrial siphon, and an elaboration of the branchial sac so that its internal surface is increased by a series of pleats or folds. In the Styelidae (inclusive of Botryllidae) lateral or atrial budding has been acquired.

VII—THE PEROPHORIDAE

There remains to be discussed the family Perophoridae. In many respects it would seem to be but an early step in the change from the cionid to the ascidiid type, but there are indications that this is not so.

In common with the Ascidiidae the intestine has been shifted forwards along the left side of the branchial sac, together with the gonads, although the stomach lies

posteriorly to the sac as in *Ciona*. In the larger genera such as *Ecteinascidia*, internal longitudinal vessels are to be found in the branchial wall, as in *Ascidia*, *Ciona*, and *Diazona*. In the smaller *Perophora* the papillae that give rise to the vessels in larger forms are present.

If the Perophoridae do not represent a step in the trend from *Ciona* to the Ascidiidae and Stolidobranchia, then they must have evolved from the stock of those forms in which the viscera have descended the stalk. For this there is some evidence. There are two valves between the posterior end of the stomach and the hind part of the intestine that suggest forcibly that the gut-loop once occupied the stalk as in *Distaplia* (cf. *Ecteinascidia* and *Distaplia*, etc., in figs. 7 and 13). The oviduct is short and wide and barely reaches the base of the atrial chamber, a condition typical only of the smaller types with descended viscera. There is a hypertrophied stolon vessel with a mesenchymatous septum. Buds develop from the stolon as in *Clavelina* and *Colella*, although without fragmentation or isolation from the parent zooid. Lastly, there is no trace of the epicardium either in its original form or as a renal vesicle at any stage of development.

Since the ventral test vessel with its septum is present in *Ciona*, its hypertrophy and development as a budding stolon in Perophoridae could have occurred without the descent of the viscera into the stalk.

Altogether the evidence is conflicting, and it is quite possible that the Perophoridae may represent an evolution from the primitive cionid stock independently of the two major trends of descending and ascending viscera described above.

The Perophoridae illustrate again the relation between body size and branchial structure, and the danger of placing great emphasis on the absence of longitudinal vessels. *Perophora* and *Ecteinascidia* are very closely related, yet the dwarfing that is evident in the former has resulted in the non-development of those vessels. Similarly, while branchial folds are the main characteristic of

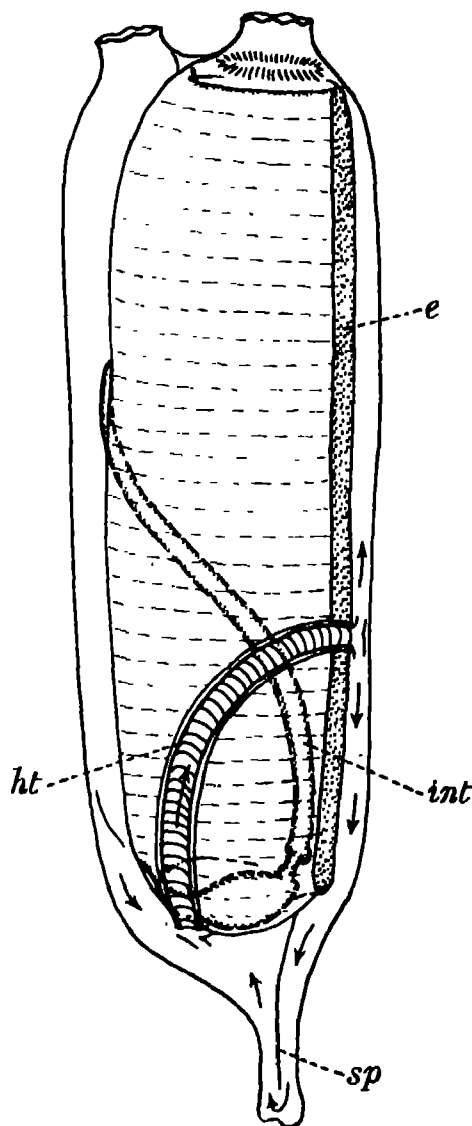


FIG. 13—Adult morphology of *Ecteinascidia turbinata*, showing translocation of heart and intestine. *e*, endostyle; *ht*, heart; *int*, intestine; *sp*, mesenchymatous septum.

the order Stolidobranchia, they are absent in the dwarfed botryllids, *Symplegma* and in *Eugyra*.

It is considered, moreover, that while the Stolidobranchia (Ptychobranchia) form a natural order or suborder, the Phlebobranchia (Dictyobranchia) are very heterogeneous and that certain members of this order are more closely related to members of the Aplousobranchia (Krikobranchia) than to other members of their own order.

It is proposed, therefore, to construct a classification based more upon the organization as a whole, and especially upon structure less likely to have undergone convergent evolution and less dependent upon mere body size than that of the branchial sac.

VIII.—CLASSIFICATION

The various regions, organs or structures of ascidians upon which classification may be based are principally the branchial sac, the post-branchial intestine, the gonads, the heart and the epicardium. Of these, objections have already been raised against the first. The relative position and the appearance of the post-branchial intestine or gut-loop is not entirely satisfactory as a basis, and in any case does not present sufficient variability. The same applies to the gonads, since they seem to have certain positional relationships to the intestine, and also are influenced structurally to some extent by the size of the individual. The heart again exhibits insufficient variation, and, while all the above-mentioned structures are of great diagnostic value when taken together, the epicardium alone as a single organ seems to reflect the major divisions of the class.

The epicardium will accordingly be used as the basis for separating the class into its orders, the other structures for dividing those orders into families and sub-families. Inasmuch as the epicardia, in their development and general nature, seem more than reminiscent of the vertebrate coelom, the order names will be based upon this similarity.

Whether or not *Ciona* be as primitive as suggested earlier, there is no doubt that it stands apart from other ascidians, and inasmuch as the epicardia are believed to exist in this genus in their most primitive form, it is suggested that the genus comprises in itself the order DIPLOCOELA.

In contrast to the unique structure of *Ciona*, there are innumerable forms in which the epicardia have descended the stalk with the gut-loop and have lost the openings into the pharynx. With the exceptions of *Diplosoma* and *Euherdmania* the two chambers have fused, and these two exceptions are by no means necessarily primitive retentions. This large group is designated as the order EPICARDIOCOELA.

In the Perophoridae the chambers have apparently been lost entirely and the family in many ways stands apart from other ascidian types. In consequence it is felt justifiable to construct a separate order for its reception, namely, the ACOELA.

The remaining ascidians, the Ascidiidae, Rhodosomatidae, and the families of the order Stolidobranchia (Ptychobranchia) comprise a natural group so far as

the epicardia form, at some stage of development, a closed vesicle containing a renal concretion. The excretory function of the epicardia is thus emphasized, and it is proposed to include all these families within the order NEPHROCOELA.

Of the above orders, the Diplocoela and the Acoela are so small that no subdivision of the order and single family in either is necessary or desirable. In the Epicardiocoela and Nephrocoela the need is great.

The Epicardiocoela contain the old families Diazonidae, Synoicidae, Didemnidae and Clavelinidae (including Distomidae). Of these the first three are fairly well defined, but the last is decidedly heterogeneous. It is considered that the distomids, *Archidistoma* and *Eudistoma* are virtually dwarfed diazonid types, and the family Diazonidae will be re-defined to include these genera and to exclude *Tylobranchion*, a genus resembling *Diazona* rather than the Synoicidae only in the presence of branchial papillae. The Synoicidae will be enlarged to include all Epicardiocoela with post-abdominal extensions containing heart, epicardia, gonads, and muscle, namely, the Synoicidae plus *Tylobranchion* and *Euherdmania*, although these may represent three lines of parallel evolution from a diazonid stock. In view of this possibility and of certain distinguishing features between the three groups, they will be defined as subfamilies. It is of interest that there is a striking resemblance between the tadpole larvae of *Eudistoma* and those of the Synoicidae, suggesting the origin of the last group from the former.

The genera *Clavelina*, *Chondrostachys*, *Sigillina*, *Podoclavella*, *Colella*, and *Distaplia* are all characterized by a hypertrophy of the ventral stolon vessel and by a correlated straightening-out of the originally V-shaped heart. *Pycnoclavella* in some ways seems to be intermediate in type between the above and the *Eudistomid*, although it is also highly specialized. For convenience it is included with the above as part of the family Clavelinidae. This family remains somewhat heterogeneous, and, on the basis of mode of budding, could be divided into four sub-families.

The remaining family of this order, the Didemnidae, has no need of modification. The nature of the heart, general organization, and method of budding suggest a relationship with *Distaplia*, the enlarged ventral stolon having been lost entirely.

Thus the Didemnidae and Clavelinidae are connected by the nature of the heart, and so are the Diazonidae and Synoicidae. To indicate these relationships, the Epicardiocoela is divided into two sub-orders, the Dicardia containing the two last-mentioned and the Unicardia the first two families.

The Nephrocoela must also be divided into two sub-orders, one to include the families Ascidiidae and Rhodosomatidae, the other the Styelidae, Botryllidae, Pyuridae, and Molgulidae. In current classifications these two groups are separated by virtue of differences in the branchial sac, the latter alone having branchial folds. Since, however, dwarfed types of this last are without folds, it is preferred to base the separation upon other characters, and with this object names are revived from Perriers's classification, Enterogona and Paragona. In the sub-order Enterogona the gonads occur only on one side of the body and within the primary loop of the gut. In the Paragona they occur on each side of the body and with no fixed

relation to the primary gut-loop on that side. Reasons for the subordination of the Botryllidae within the Styelidae have been given elsewhere (BERRILL, 1932).

The full classification as indicated above, together with brief definitions of the various groups, is given below.*

CLASS ASCIDIAGEA

Order	Sub-Order	Family	Sub-Family	Genera
1. DIPLOGOELA (Epicardia in form of two perivisceral sacs opening anteriorly into lumen of pharynx.)		<i>Cionidae</i>		<i>Ciona</i>
2. EPICARDIOCOELA (Epicardia descended into stalk with rest of viscera, openings to pharynx lost, and the two chambers usually fused.)				
	A. DICARDIA (Heart V-shaped with raphe not closed by epicardium.)			
		<i>Diazonidae</i> (No post-abdomen, budding by abdominal constriction.)		<i>Rhopalea</i> <i>Diazona</i> <i>Archidistoma</i> <i>Eudistoma</i>
		<i>Synoidae</i> (Post-abdomen, budding by post-abdominal constriction.)		
			<i>Synoidae</i>	<i>Morchellium</i> <i>Sidnyum</i> <i>Polyclinum</i> <i>Amaroucium</i> <i>Aplidium</i>
			<i>Euherdmanias</i>	<i>Euherdmania</i>
			<i>Tylobranchionae</i>	<i>Tylobranchion</i>

* The classification given here is to be regarded as tentative and not in its final form.

CLASS ASCIDIACEA—(continued)

Order	Sub-Order	Family	Sub-Family	Genera
	B. UNICARDIA			
	(Heart straight with raphe closed by epicardium.)			
		<i>Clavelinidae</i> (Ventral sto- lonic vessel enlarged.)		<i>Archiascidia</i> <i>Clavelina</i> <i>Podoclavella</i> <i>Chondrostachys</i> <i>Sigillina</i> <i>Colella</i> <i>Distaplia</i> <i>Pycnoclavella</i>
		<i>Didemnidae</i> (Ventral sto- lonic vessel absent.)		<i>Didemnum</i> <i>Diplosoma</i> <i>Coelocormus</i>
3. ACOELA (Without trace of epicardia.)		<i>Perophoridae</i>		<i>Perophora</i> <i>Perophoropsis</i> <i>Ecteinascidia</i>
4. NEPHROGOELA (Epicardia in form of renal vesicle or vesicles.)				
	A. ENTEROGONA			
	(Gonads on one side of body only.)			
		<i>Asciidiidae</i>		<i>Ascidia</i> <i>Ascidiella</i>
		<i>Rhodosomatidae</i>		<i>Rhodosoma</i> <i>Chelyosoma</i> <i>Corella</i>
	B. PARAGONA			
	(Gonads on each side of body.)			
		<i>Styelidae</i>		<i>Styela</i> <i>Polycarpa</i> <i>Styelopsis</i> <i>Polyandrocarpa</i> <i>Distomus</i> <i>Alleocarpa</i> <i>Stolonica</i> <i>Symplegma</i> <i>Botryllus</i> <i>Botrylloides</i>

CLASS ASCIDIACEA—(continued)

Order	Sub-Order	Family	Sub-Family	Genera
		<i>Pyuridae</i>		<i>Pyura</i> <i>Tethyum</i> <i>Boltenia</i> <i>Culeolus</i> <i>Microcosmus</i> <i>Forbesella</i>
		<i>Molgulidae</i>		<i>Molgula</i> <i>Eugyra</i>

A COMPARISON OF CURRENT AND PROPOSED CLASSIFICATIONS

Classification of LAHILLE and of SEELIGER		Proposed Classification	
Order	Family	Sub-Order	Order
STOLIDOBRANCHIA or PTYCHOBRANCHIA	Molgulidae Pyuridae Styelidae Botryllidae	Paragona	NEPHROGOELA
		Enterogona	
PHLEBOBRANCHIA or DICTYOBRANCHIA	Ascidiidae Rhodosomatidae Perophoridae Cionidae Diazonidae		AGOELA DIPLOGOELA
APILOUSOBRANCHIA or KRIKOBRANCHIA	Synoicidae Distomidae Didemnidae Clavelinidae	Dicardia Unicardia	EPICARDIOGOELA

While the above classification is based primarily upon the nature of the epicardia, and partly also on the heart, a grouping of the ascidians according to their mode of budding (*cf.* BERRILL, 1935, *b*) would approximate closely to the same scheme. The difference between that proposed and the current classifications is that the order Phlebobranchia (Dictyobranchia) is regarded as most heterogeneous and has been split so that two families, the Cionidae and Perophoridae, become two orders, and the remaining families are placed among the other two orders, the Stolidobranchia and Aplousobranchia, the enlargement of which necessitates re-definition.

It has been stated already that since there is a correlation between the body size of an individual and the presence or absence of branchial papillae, and also between size and the fusion of papillae to form inner longitudinal vessels, that such differences

are of questionable value when used for the distinction of orders, as in distinction between the Phlebobranchia (Dictyobranchia) and the Aplousobranchia (Kriko-branchia). This objection is supported by the fact that distinction itself is not exact, for in the Synoicid genera *Polyclinum* and *Glossophorum*, both with relatively large branchial sacs, papillae are definitely present.

Apart from this relegation of branchial structure to a place of secondary importance, the classificatory scheme proposed agrees with the more general conception of ascidian relationships.

In fig. 14 is summarized diagrammatically the interrelationships of the various orders, families and genera.

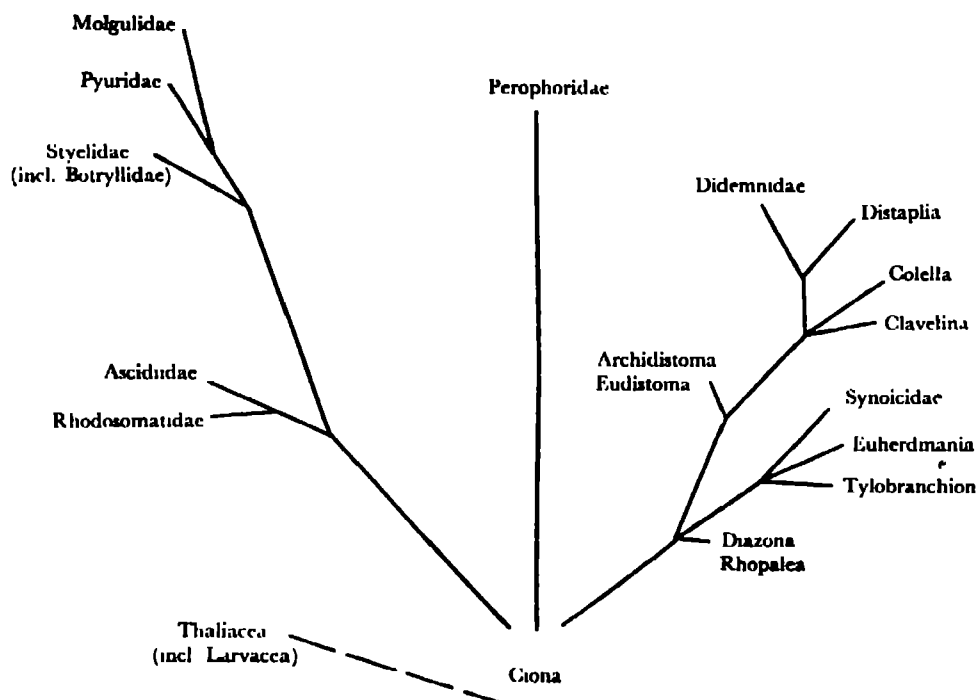


FIG. 14.—Diagram of probable interrelationships of ascidian families.

IX—THE THALIACEA AND LARVACEA

If the foregoing classification reflects in any way the evolutionary trends within the Ascidiacea, it should be possible to relate to them in some way the evolution or origin of the Thaliacea (*Pyrosoma*, *Doliolum* and *Salpa*) and Larvacea. In a recent paper GARSTANG (1928) has assembled evidence to show that the Larvacea, far from being surviving primitive ancestral forms as commonly supposed, are in reality highly specialized neotenous doliolids. With this contention the writer is in full agreement, and the development of *Oikopleura* recorded by DELSMAN (1912), though not considered by GARSTANG, is further indication of an extreme specialization rather than a primitive nature of these forms.

The Thaliacea as a whole evidently have originated from a single stock, as evidenced by their peculiar mode of budding from an epicardial outgrowth at the base of the endostyle. In all three thaliacean families the branchial sac, atrium and musculature are highly specialized, and are obviously related to the demands of a free-swimming pelagic life.

The gonads are single and lie close to the loop of the intestine, and this fact, in the absence of positive evidence to the contrary, precludes relationship with the *Paragona* (Stolidobranchia). The presence of epicardia excludes close affinity with the Acoela (Perophoridae), in spite of some resemblance in the methods of budding. At the same time, no structure in the Thaliacea suggests that the viscera have ever descended a fixation stalk as in the Epicardiocoela (Diazonidae plus Aplousobranchia), and the position of the post-branchial gut and the heart resembles that in newly metamorphosed individuals of *Ciona*, *Diazona*, or *Ascidia*. This relative position of gut and heart to the rest of the body is, in fact, extremely primitive. In young forms the epicardia retain their openings with the pharynx, although the distal epicardial tissue is specialized for budding and does not form the lining of the perivisceral cavity as in *Ciona*.

Thus, of the various types of ascidians, the Thaliacea can be related only with *Ciona*, and the evidence even in this case is merely negative, suggesting that the Thaliacea may have evolved at almost any stage during the evolution of the stock that culminated in *Ciona*.

X--FUNCTION OF THE EPICARDIUM

The epicardium obviously has a variable function among ascidians. In *Diplosoma* and in the Thaliacea it is without doubt concerned primarily with the formation of totipotent strands in connexion with regeneration and budding. In the Molgulidae and some other families it is equally certain that the epicardium has no concern with budding and is primarily an organ of excretion. It would seem likely, therefore, that the two functions just described are two extreme specializations of the epicardium and that primitively both functions might be present in a less spectacular form. Secretions into the lumen in the Epicardiocoela suggest an excretory function, especially as the epicardium is always in close association with the pericardium even in the Molgulidae, while the secondary apertures into the atrial cavity in *Diazona* reinforces this suggestion. At the same time, in many of these forms the epicardial lining epithelium is the source of all new cells during regeneration or budding. This, however, merely denotes the absence of any significant or irreversible specialization, for totipotency is characteristic of other tissues that have normally a comparatively passive function. Thus the cells of the mesenchymatous septum of the vascular stolon are also totipotent, although the septum has no developmental or other connexion with the epicardium, as has been commonly believed. It arises from the base of the pericardium even in the oozoid of *Clavelina*, the one case where it has been stated to be of epicardial origin,

and it seems to be merely a septum of mesenchyme induced by vascular exigencies. Other totipotent cells form the lining of the atrial cavity, as is shown by budding in the Styelidae.

Thus from the point of view of function, the epicardium seems to be most primitive again in *Ciona*. There it allows water drawn into the branchial chamber to circulate slowly around the heart, intestine and gonads, and yet in the rare occurrence of the loss of anterior tissues in this genus, the new formative cells arise from the epicardial lining. There is therefore some suggestion of excretory function of the epicardium in *Ciona* and definite evidence of an unspecialized condition of the cells forming it. In many ways, in fact, the ascidian epicardium as it appears in *Ciona* resembles the coelom of the higher chordates. This question will be considered more fully elsewhere.

XI—SUMMARY

An account is given of the adult morphology of the heart and epicardium in the following genera: *Ciona*, *Diazona*, *Rhopalea*, *Tylobranchion*, *Euherdmania*, *Sidnyum*, *Eudistoma*, *Archidistoma*, *Distaplia*, *Colella*, *Diplosoma*, *Pycnoclavella*, *Clavelina*, *Perophora*, *Ecteinascidia*, *Ascidia*, and *Molgula*.

The development of the heart and epicardium is described for *Ciona*, *Diazona*, *Ascidia*, *Polycarpa*, and *Molgula* (i.e., genera all with small eggs).

The heart develops as an infolding of the pericardium, which in turn is an evagination from the base of the pharynx. At first it is a straight tube opening at one end in the region of the stomach, at the other into the subendostylar vessel at the base of the endostyle. With growth of the individual in *Ciona* the heart becomes V-shaped as the result of extension in length between two relatively fixed points. In *Diazona*, and in the synoicids and distomids, it sinks into the stalk together with the rest of the viscera, the bend of the heart resting in the base of the stalk. In the clavelinids and certain distomids hypertrophy of the ventral or posterior stolonial vessel has had the effect of shortening and straightening the heart, so that the end which primitively opened near the stomach now opens posteriorly. In the Perophoridae the heart has become dissociated from the rest of the viscera and extends as a long tube along one side of the branchial sac, the end that opens into the subendostylar vessel having become shifted anteriorly. In the Ascidiidae, Styelidae, Pyuridae, and Molgulidae, a similar shifting has occurred, although the relative displacement of the heart along one side of the branchial sac is less striking than the shifting of the intestinal loop forwards along the other side.

The epicardium in *Ciona* and *Diazona* develops as a pair of evaginations from the posterior end of the pharynx. In *Ciona* these grow and envelop the viscera, maintaining open communication. In *Diazona* the openings are lost, the two sacs fuse to form a single chamber between the heart and intestinal loop, and descends with the viscera as a whole into the stalk, similar to the conditions in the Synoicidae, Didemnidae, Distomidae, and Clavelinidae. In the Perophoridae there is no sign of the epicardium in the adult or at any stage of development.

In *Ascidia*, *Polycarpa*, and species of *Molgula* it develops as a median evagination from the posterior end of the pharynx, becomes a closed vesicle, and contains a renal concretion. Such vesicles become numerous in adult *Ascidia*, disappear in *Polycarpa*, but remain recognizable and single in Molgulids and the pyurid *Microcosmus*.

Evidence is cited to show the doubtful value of branchial structure as a basis for classification, and the relationship between such structure and the absolute size of an ascidian.

A new classification of the class Ascidiacea is proposed, based primarily upon the nature of the epicardium. The result has been mainly to break up the heterogeneous order Phlebobranchiata (Dictyobranchiata), and to form five orders in place of three. It is believed that the classification proposed is a more probable reflexion of the course of evolution within the Ascidiacea than those displaced.

It is suggested that the function of the epicardium is to be found in its most primitive condition in *Ciona*, where it is of a coelomic nature, partly excretory, but so unspecialized that its cells can play a formative part in regeneration of missing parts. It is believed that the importance of the epicardium in the budding processes of such forms as *Diplosoma*, *Aplidium*, and the Thaliacea, and as an excretory organ in the Ascidiidae, Styelidae, Pyuridae, and Molgulidae is the result of extreme specialization in different directions of functions present, but poorly developed, in *Ciona*.

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previous litters. It is probable, in fact, that the majority of animals become pregnant again at the post-partum oestrus. Thus, those which start to breed at the beginning of the season and continue until the end of July or beginning of August may rear as many as four or even five litters in succession. The frequent occurrence of pregnant animals that were also lactating confirms these deductions. In some of the early stages of pregnancy the condition of the uterus as well as that of the mammary glands showed that the pregnancy dated from an oestrous period occurring immediately after parturition.

The mean number of young in a litter can be estimated from the mean number of ova ovulated at each oestrus as shown by the number of corpora lutea in each set, from the mean number of embryos *in utero* in the later stages of pregnancy and from the mean number of placental sites visible in parous uteri.

Analysis of the number of corpora lutea in each set and their distribution yields interesting information. The total number of complete sets of corpora lutea available was 277. These were distributed as in Table IV. It can be seen that the most frequent number of ova ovulated at one oestrus was 4, that the largest number observed was 12, and the mean number 4.43. The percentage frequency of each class is represented graphically in fig. 4.

TABLE IV

No. in set or litter	Observed			Compiled from ADAMS and BAKER. Embryos
	Corpora lutea	Placental sites	Embryos	
1	1	—	1	1
2	8	1	1	4
3	43	11	16	29
4	108	20	27	35
5	82	13	21	14
6	22	11	4	5
7	7	2	—	—
8	2	—	—	—
9	2	—	—	—
10	—	—	—	—
11	1	—	—	—
12	1	—	—	—
Total . . .	277	58	70	88

The distribution of the corpora lutea in each set between the two ovaries of each pair, represented as the difference between the numbers in each ovary, is shown in the third column of Table V. The expected values, on the assumption that the distribution between the two ovaries is random, are given in the second column and the divergences of the observed from the expected values in the fourth column.

Testing by means of χ^2 (FISHER, 1930) the divergence of the observed from the expected values is found to be without significance, and therefore the distribution of

the corpora lutea of each set between the two ovaries of a pair may be assumed to be random.

TABLE V

Difference between the number of corpora lutea in each of a pair of ovaries	Expected	Observed	Divergence of observed from expected values	χ^2/m
5+	7.5	5	- 2.5	0.833
4	18.3	22	+ 3.7	0.748
3	39.7	25	- 14.7	5.443
2	69.6	70	+ 0.4	0.002
1	89.8	106	+ 16.2	2.922
0	52.1	49	- 3.1	0.184
Total	277.0	277	0.0	$\chi^2 = 10.132$

The number of embryos in each of 70 late pregnancies and the number of placental sites in each of 58 parous uteri are given in the third and fourth columns of Table IV. It will be seen that the largest number in a uterus was 6 for the embryos and 7 for the placental sites. The mean number of embryos was 4.11 which is rather greater than 3.82 recorded independently by both ADAMS (quoted by BARRETT-HAMILTON, 1911) and BAKER (1930) whose data are given in the fifth column. The mean number of placental sites is 4.48. The numbers of embryos and placental sites in the litters observed are represented as percentage frequencies in figs. 5 and 6, which are therefore comparable to fig. 4.

The number of ova ovulated at each oestrus exhibits considerable seasonal variation, as shown by the data given in Table VI.

TABLE VI

Month	No. of animals	No. of sets of corpora lutea	Total No. of corpora lutea	Mean No. of corpora lutea in a set
April	28	46	180	3.9
May	78	109	519	4.8
June	38	47	235	5.0
July	24	31	132	4.3
August	33	40	146	} 3.6
September	3	3	10	
October	1	1	4	
Total	205	277	1,226	4.43

The mean numbers of corpora lutea in a set, for each period, as shown in the last column of Table VI, are represented graphically in fig. 7.

The number of ova ovulated at each oestrus varies also according to the body-weight, as is shown by the data presented in Table VII. It can be seen from these data that the number of ova ovulated is proportional to the body-weight.

TABLE VII

Body-weight groups, gm	Mean body-weight, gm	No. of animals	No. of sets of corpora lutea	Total No. of corpora lutea	Mean No. of corpora lutea in a set
26<	28.6	35	44	227	5.2
24-25.9	24.9	25	35	164	4.7
22-23.9	22.8	27	34	158	4.6
20-21.9	20.9	41	60	268	4.5
18-19.9	18.8	38	59	235	4.0
>17.9	15.6	28	32	117	3.7
Total . . .	21.9	194	264	1,169	4.43

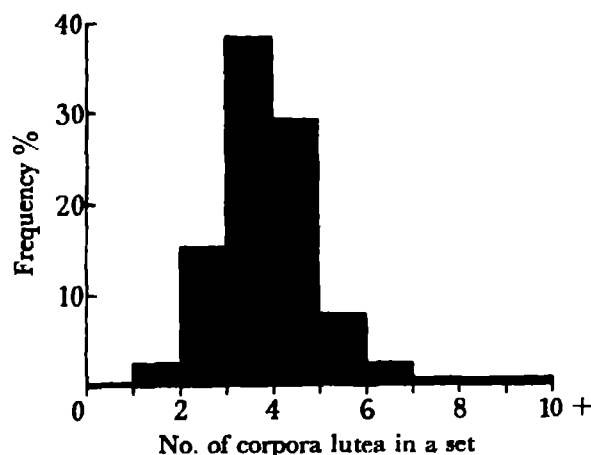


FIG. 4.

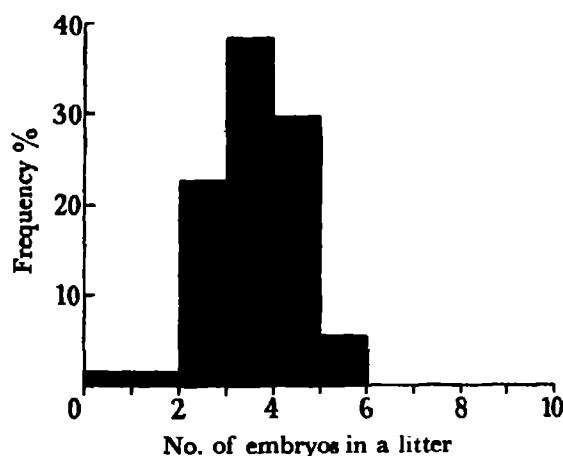


FIG. 5.

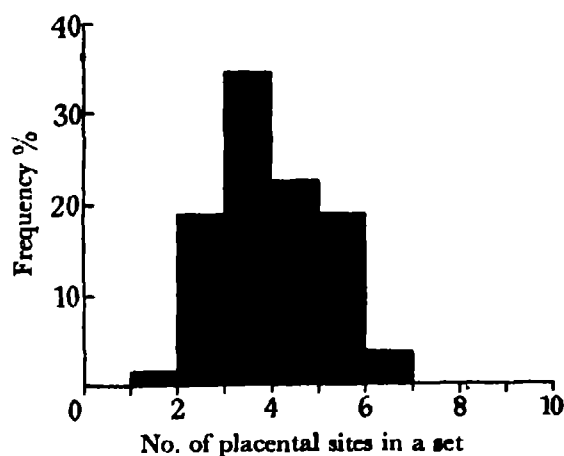


FIG. 6.

FIGS. 4-6—Frequency polygons illustrating size of litter as shown by the number of corpora lutea in a set, fig. 4; by the number of embryos *in utero* in a litter, fig. 5; and by the number of placental sites in a pair of post-partum uteri, fig. 6. The frequency in each case is represented as a percentage of all cases to render the figures more readily comparable.

Statistical examination of the whole of the data given in this table shows that they can be represented in the form of a straight line regression of number of corpora lutea in a set on body-weight of the form :—

$$y = 0.1144x + 1.92,$$

where y = the number of corpora lutea in a set, and x = the body-weight. Testing by means of the table of t (FISHER, 1930) this regression is found to be highly significant. The regression line and the means of each group are represented graphically in fig. 8.

Since the body-weight varies seasonally, reaching a maximum in June at the end of the spring rise and falling thereafter, owing to the appearance of young animals and possibly to an actual decrease in weight of the old animals, it is clear that the relations of the number of ova ovulated to month on the one hand and to the body-weight on the other hand cannot be considered as independent. If it is assumed that the seasonal variation is the more fundamental, then the relation to body-weight would follow in consequence to a large extent. Conversely if the relation to body-weight is the more fundamental then it would largely explain the seasonal variation. Unfortunately the data do not permit of deciding which relation is fundamental. It may be significant in this connexion that the pituitary itself is known to exhibit a positive heterogonic relation to body-size in the rabbit (ROBB, 1928, 1929, and ALLANSON, 1932). If the amount of hormone produced by the pituitary is proportional to its size then positive heterogony of the pituitary would account for the number of ova ovulated being proportional to the body-size.

The number of embryos *in utero* in late stages of pregnancy also varies seasonally and with body-weight. The data concerning seasonal variation are given in Table VIII together with those recorded by BAKER (1930) for the seasons 1926 and 1927.

TABLE VIII

Month	Observed			Compiled from BAKER (1930)		
	No. of litters	No. of embryos	Mean size of litter	No. of litters	No. of embryos	Mean size of litter
Before June	31	134	4.3	22	85	3.9
June and July	27	113	4.2	17	75	4.4
After July	12	41	3.4	11	31	2.8
Total	70	288	4.11	50	191	3.82

The data according to body-weight are given in Table IX.

TABLE IX

Body-weight groups, gm	No. of litters	No. of embryos	Mean size of litter
25 <	15	67	4.5
20-24.9	34	142	4.2
> 19.9	13	50	3.8
Total	62	259	4.18

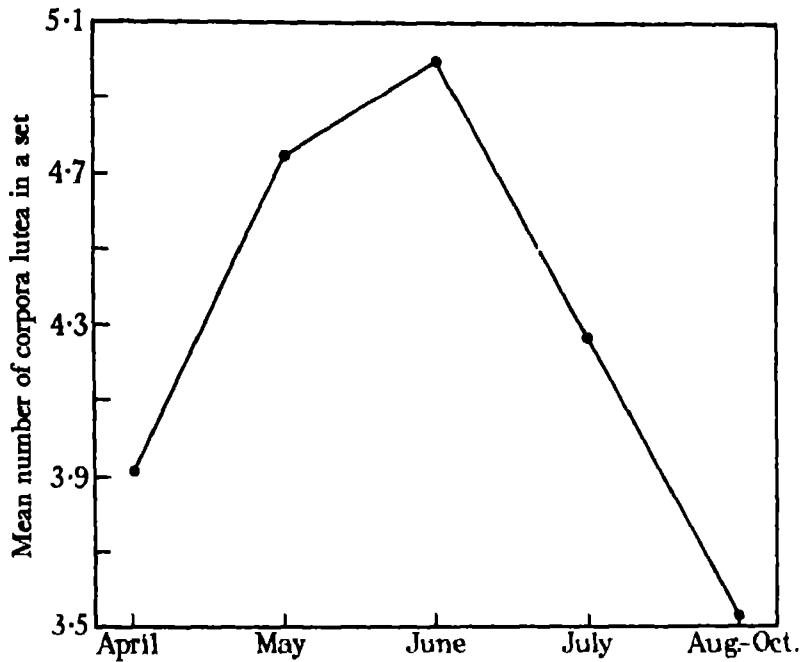


FIG. 7—Graphical representation of the mean number of corpora lutea in a set according to the month. The data are given in Table VI to the nearest first decimal place.

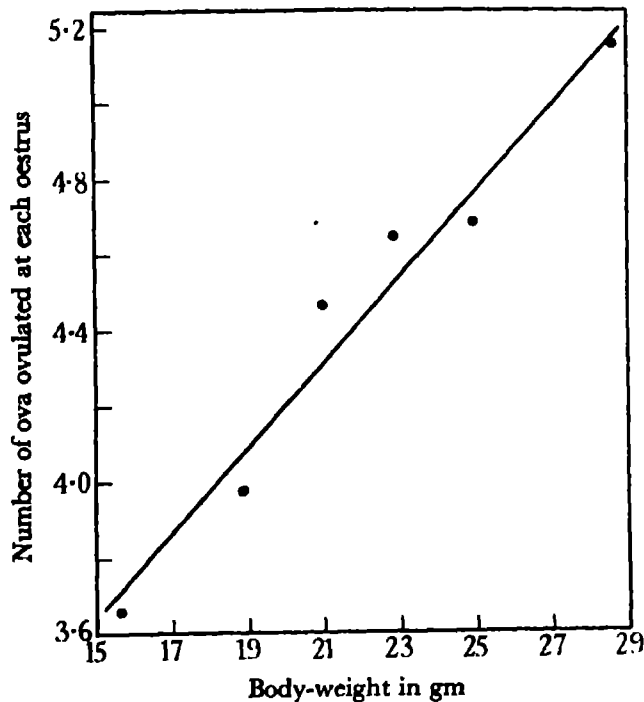


FIG. 8—Graphical representation of the regression of the number of ova ovulated on body-weight. The points represent the mean values for the body-weight groups given in Table VII to the nearest first decimal place, and are plotted for comparison with the calculated regression line.

The body-weights of those animals which were in late stages of pregnancy were corrected by subtraction of the weights of the uteri and contained embryos for the purposes of this table.

The observations show that the number of embryos *in utero* falls off as the season advances, although BAKER's somewhat less extensive data show a maximum in June and July. The number of embryos *in utero* increases with body-weight.

Information regarding the incidence of intra-uterine mortality is provided by comparison of the number of embryos with the number of ova ovulated as shown by the corpora lutea in late uterine pregnancies. The data, grouped according to the number of ova ovulated, are given in Table X. It can be seen that there is a much heavier mortality, as judged both by the percentage of litters affected and the percentage of ova lost, when 6 or more, than when 5 or less, ova are ovulated. Moreover, in no case have more than 6 survived (*see also* fig. 5). It is reasonable to assume that this mortality is not due to genetical causes, since these would not explain the differential incidence observed.

TABLE X

No. of ova ovulated	No. of examples	No. of animals showing mortality	No. of ova lost	% of animals showing mortality	% of ova lost
2	1	0	0	20.7	6.6
3	8	1	2		
4	28	7	8		
5	21	4	6		
6	9	6	10	75.0	24.5
7	1	1	2		
8	1	1	4		
9	1	1	3		
Total . . .	70	21	35	30.0	10.9

The increased mortality when 6 or more ova are ovulated might be due either to deficiency of nutritive and other substances necessary for the maintenance of the embryos or to the mechanical effects of overcrowding in one or both of the uterine cornua. If the latter alternative were true the mortality, falling most heavily on the cornu with the larger numbers of embryos would result in a tendency towards equality in the distribution of the healthy embryos between the two cornua. This tendency should be detected by comparing the observed distribution of embryos in late pregnancies and of old placental sites in parous animals with the expected random distribution as given in Table XI. The agreement of observed and expected values is very close, and when tested by means of χ^2 (FISHER, 1930) there is obviously no significant departure from the random distribution.

Examination of the mortality in each uterine cornu according to the number of ova ovulated in the corresponding ovary without reference to the other side, that is

TABLE XI

Difference between No. in right and left uterine cornua	Expected	Observed		Divergence of sum of the observed from expected values	χ^2/m
		Embryos	Placental sites		
5+	2.7	2	1	+0.3	0.225
4	8.7	5	5	+1.3	
3	18.1	10	5	-3.1	0.531
2	31.6	14	16	-1.6	0.081
1	43.6	26	20	+2.4	0.132
0	23.3	13	11	+0.7	0.021
Total . . .	128.0	70	58	0.0	$\chi^2 = 0.990$

without taking account of the total number of ova ovulated in the two ovaries, supports this conclusion. The data are given in Table XII.

TABLE XII

No. of ova ovulated in each ovary	No. of ovaries	Total No. of ova ovulated	No. of ova lost	% of ova lost
1 or 2	73	125	13	10.4
3 or more	52	183	15	8.2

It is apparent that the mortality is actually higher, though not significantly, when 1 or 2 than it is when 3 or more ova are ovulated in one ovary. It is concluded that the increased mortality when the total number of ova ovulated from both ovaries is large, is due to deficiency of nutritive or other substances required for development and not to mechanical effects of overcrowding or failure of the uterus to contain the embryos.

VII—OESTROUS CYCLE

a. Dioestrous Cycle

The histological changes in the reproductive organs during the oestrous cycle are so similar to those of the white mouse and the rat that they do not require detailed description. It will be sufficient for the purposes of this paper to refer to, and figure, the more characteristic phases for comparison with those of the mouse. The oestrous cycle does differ, however, from that of the white mouse in several ways, probably all connected with the fact that *Evotomys* is a wild species with a restricted breeding season, whereas the white mouse is semi-domesticated and has a continuous breeding season.

The majority of females undergo, at the beginning of the breeding season in April and May, a number of sterile cycles, accompanied by ovulation, before they become pregnant. They resemble in this respect the Hedgehog in which DEANESLY

(1934) has recorded the occurrence of a number of sterile dioestrous cycles accompanied by ovulation. This is shown by the presence in the ovaries of several distinct sets of corpora lutea, sometimes as many as four, fig. 11, Plate 10. Since it is very doubtful whether corpora lutea would persist long enough to be recognizable after more than four dioestrous cycles it is quite possible that some of the animals undergo more than four cycles before becoming pregnant. The number of non-pregnant and pregnant animals with tubal ova that had 1, 2, 3, or 4 sets of corpora lutea in the ovaries are shown in Table XIII. Pregnant animals with uterine stages are excluded because an increasing number of the earlier sets of corpora lutea would be indistinguishable as pregnancy advanced. All animals that could be in an immediately post-partum or post-lactation oestrus are omitted also, since they would show the corpora lutea of pregnancy or lactation respectively, as well as those of the subsequent oestrus.

TABLE XIII

No. of distinct sets of corpora lutea in the ovaries	April and May		June to October	
	Non-pregnant	Pregnant	Non-pregnant	Pregnant
1	2	1	1	5
2	7	10	1	1
3	5	17	—	1
4	1	6	—	1

It will be seen that only one of the 34 tubal pregnancies obtained in April and May which are recorded in Table XIII became pregnant at the first oestrus. One animal (E 520, *see* Table XV, and fig. 12, Plate 10), in addition, was obtained which was in its first oestrus but had not yet ovulated. The ova in the large follicles about to ovulate contained 1st polar spindles, and the animal had mated as shown by the vaginal plug still *in situ*. It may be concluded that although pregnancy begins most frequently at the third oestrus it may do so even at the first or not until the fifth.

There is no evidence that any of these sterile cycles were accompanied by copulation. Spermatozoa were not found in any non-pregnant animals except in those in oestrus which had obviously just mated. No animals were found which were pseudo-pregnant. It seems probable, therefore, that these sterile cycles are not accompanied by copulation and that the latter, when it does occur, is usually fertile. The cause of this failure to copulate at these early oestrous periods is obscure, but it lies, presumably, in the state of the female since the males are all in full breeding condition at this time.

Young animals which attain puberty in the latter part of the season appear to become pregnant at the first oestrus much more frequently, as is shown by the data in the fourth and fifth columns of Table XIII. This difference between animals passing from winter anoestrus into the breeding condition and animals attaining puberty during the breeding season is remarkable.

It seems probable that the sterile dioestrous cycle is of very short duration because of the sudden appearance of animals with several sets of corpora lutea at the onset

III—Reproduction of the Bank Vole (*Evotomys glareolus*, SCHREBER)*

I—The Oestrous Cycle of the Female

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(Communicated by A. S. PARKES, F.R.S.—Received July 26, Read November 14, 1935)

[PLATES 10 and 11]

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I—INTRODUCTION

The Bank Vole is one of the commonest and most widely distributed of British mammals, sometimes increasing in numbers to such an extent as to constitute a serious agricultural pest. The details of its reproductive processes therefore are of some economic importance to agriculture and forestry.

* Since this paper went to press the British Museum have published a "List of British Vertebrates", in which the generic name "*Clethrionomys*" is used instead of "*Evotomys*".

Remarkably little information is available regarding the reproductive processes of the species. MARSHALL (1922) states that it "is almost certainly polyoestrous, since it can become pregnant immediately after parturition at certain times of the year"; a conclusion which is fully substantiated. The results of the earlier observations on the breeding habits are summarized by BARRETT-HAMILTON (1911). Recently BAKER (1930) has provided some further information on this species, and SVIHLA (1929) has recorded some interesting observations on the breeding in captivity of the allied American species *Evotomys gapperi*.

The oestrous cycle of the Bank Vole described in this paper is that of a wild species, uninfluenced by captivity or domestication. Comparison with the oestrous cycle of the white mouse which is essentially similar in its main features should throw some light on the modifications resulting from domestication.

II—TECHNIQUE

The account of the reproductive processes of the Bank Vole contained in this and the succeeding paper is based on wild specimens none of which was kept in captivity. All the material was obtained by trapping, chiefly dead in break-back traps but part of it alive in box-traps. The greater part was obtained in North Wales but some of the animals were trapped in the Home Counties. It was collected at the same time and in exactly the same way as the Common Shrews recently described by one of us (BRAMBELL, 1935). *Evotomys* was caught slightly more frequently than *Sorex araneus* on the mainland of North Wales but was decidedly less plentiful in Anglesey where the ratio was approximately 3 Shrews to 1 Bank Vole. The technique of dissection and the histological treatment employed were similar to those described in the paper referred to on the Shrew. Complete serial sections were made of the ovaries of all animals that were not obviously immature obtained from March to October inclusive, except for some animals trapped in September and October, 1932, after the collection for these months was already completed. Since the reproductive organs of these were not preserved they are only included in this series for body-weight and sex-ratio purposes. The ovaries of some of the animals obtained during the months of November, December, January, and February were also sectioned. Complete serial sections were made of both uteri of all animals with recent corpora lutea in the ovaries that were not otherwise known to be pregnant. Counts were made of all sets of corpora lutea that could be distinguished with certainty.

III—MATERIAL AND CLASSIFICATION

The total material consisted of 1036 Bank Voles of which 443 were females. It was obtained between March, 1931, and May, 1933, in North Wales and the Home Counties. Collecting was continued systematically from March, 1931, to June, 1932. The numbers obtained each month from each county are given in Table I.

TABLE I

	Anglesey	Caernarvon- shire	Denbigh- shire	Middlesex	Kent	Essex	Total
1931							
March		4					4
April	1	3					4
June		2					2
July	2	64					66
August		37			91		128
September		30					30
October		15					15
November		11					11
December		18				1	19
1932							
January		34		1	10		45
February		59		3			62
March	13	38	10	1		2	64
April	12	66	15				93
May	5	175	4				184
June		146					146
September		46					46
October		67					67
November		30					30
December		18					18
1933							
May		2					2
	33	865	29	5	101	3	1036

The number of each sex obtained each month is given in Table II.

TABLE II

Month	♂ ♂	♀ ♀	% ♂
January	28	17	62
February	29	33	47
March	34	34	50
April	60	37	62
May	104	82	56
June	87	61	59
July	37	29	56
August	77	51	60
September	41	35	54
October	45	37	55
November	27	14	66
December	24	13	65
Total	593	443	57.24

The sex-ratio, if the whole material is to be taken as a random sample, is 57.24 ± 1.04 . BAKER (1930) records from the neighbourhood of Oxford 359 ♂ : 250 ♀, a ratio of 58.95 ± 1.34 which obviously does not differ significantly.

Classification of the material according to the stage of the oestrous cycle was based on the macroscopic condition and the histology of the reproductive organs. It was found that the oestrous cycle resembled that of the white mouse sufficiently closely to render classification relatively easy. Anoestrous animals during the autumn, winter, and early spring were identified by the inactive condition of the reproductive organs. It was usually possible to distinguish parous from non-parous anoestrous animals macroscopically by the presence of placental sites in the uterus. These retrogressed maternal placental tissues, especially the giant cells, remain for a very long time as conspicuous nodules in the mesometrial wall of the uterus. In some animals histological examination of the uterus was necessary to detect them. In a few anoestrous animals, which had no signs of placental sites, corpora albicantes, or patches of pigment in the ovarian stroma, indicated that they were parous.

During the breeding season the immature animals were readily distinguished by weight and the condition of the reproductive organs. The mature animals were divided into those with and without corpora lutea. The latter were either approaching or in their first oestrus. The former were classified as pregnant or non-pregnant animals. This involved cutting complete serial sections of the uteri and Fallopian tubes of all animals which were not obviously pregnant and searching systematically for embryos. Animals with tubal ova or unimplanted uterine blastocysts could usually be identified as parous or non-parous according to the condition of the uteri or mammary glands. In many animals, but not for all, it was possible to determine whether or not they were lactating. Late stages of pregnancy presented more difficulty in this respect and it was only possible in a few cases to be sure that they were parous and lactating. Non-pregnant animals with corpora lutea in the ovaries were found to belong to one of the following groups: (a) animals in the dioestrous cycle the stage of which was readily identified histologically, (b) parous animals in post-partum or post-lactation oestrus, and (c) animals in lactation anoestrus.

IV—STRUCTURE OF THE REPRODUCTIVE ORGANS

The reproductive organs resemble those of the mouse in their general plan, fig. 10, Plate 10. The ovaries are surrounded by closed ovarian capsules into which the Fallopian tubes open. The uterine cornua are separate and there are two distinct cervical canals opening into the vagina. The urethra runs in the ventral wall of the vagina but does not open into the vaginal lumen. The urinary opening is situated on the clitoris, thus permitting of the closure of the vaginal opening in immature and anoestrous animals. There are two pairs of thoracic and two pairs of abdominal mammae, whereas the mouse has three thoracic and two abdominal pairs.

V—GROWTH, DURATION OF LIFE, AND BREEDING SEASON

The oldest foetus obtained which, from its condition, was approximately full-term, weighed 2.37 gm when preserved. This foetus was, however, the only one in the litter and probably, therefore, was unusually large. The foetuses of another litter of four had a mean weight of 1.64 gm. Since the mean weight of these embryos in their membranes was 1.87 gm it is probable that they were also approach-

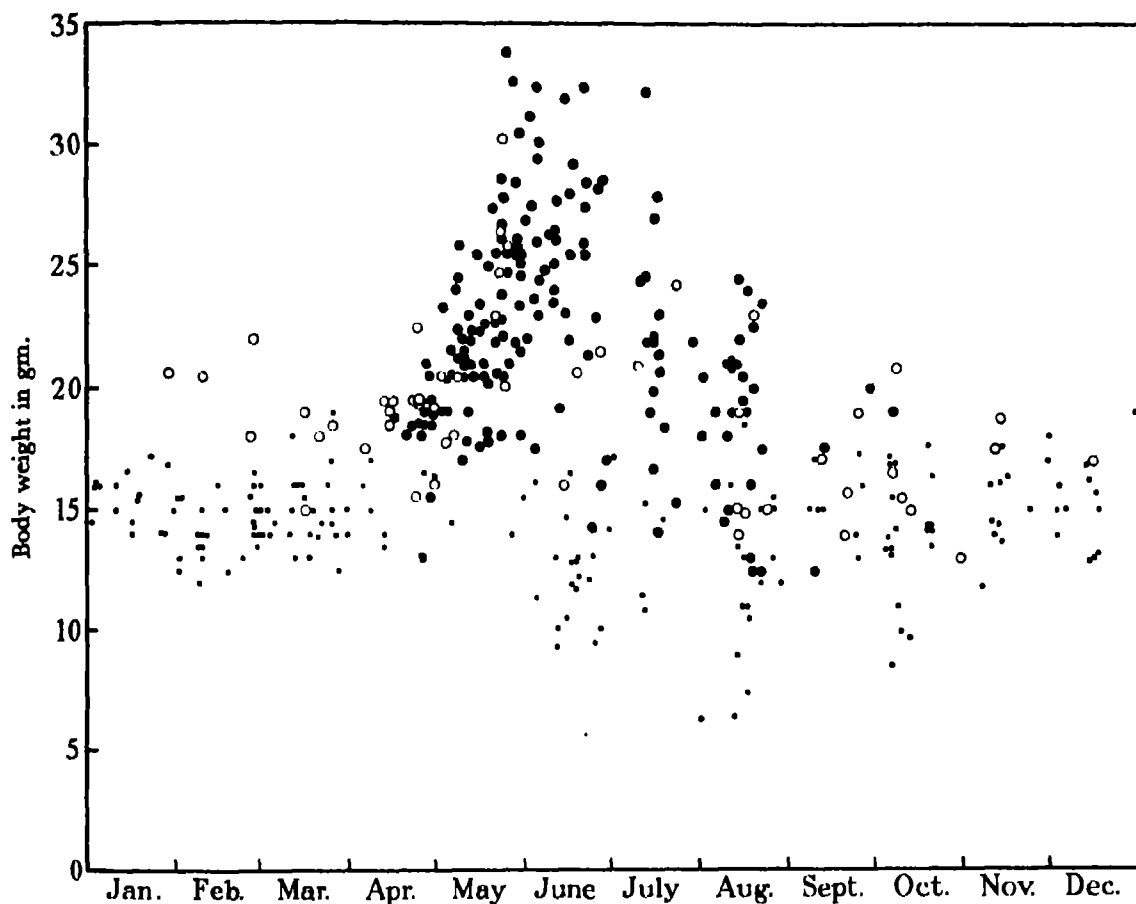


FIG. 1—Scatter diagram of the body-weights of females according to the days of the year. • non-parous non-pregnant animals; ○ parous non-pregnant animals; ● pregnant animals.

ing full-term. The estimated weight at birth is therefore approximately 2.0 gm. This figure corresponds well with those observed by SVIHLA (1929) for *E. gapperi*; he records 1.7 to 2.3 gm with a mean weight of 1.9 gm at birth.

The body-weights of 394 females are shown against the day of the year on which they were trapped in fig. 1.

It can be seen that the lightest animal weighed 6.4 gm, but that only four under 9.0 gm were obtained. Ten animals were over 30.0 gm, the heaviest being 33.8 gm, but if these weights are corrected for the foetuses and membranes of

late pregnancies only five remain over 30.0 gm, the heaviest being 32.0 gm. The lightest parous or pregnant animal was 12.5 gm, and only six were obtained under 14.0 gm. All except four of the non-parous non-pregnant animals weighed under 18 gm, and the only one of these over 19 gm was in oestrus and had mated.

All animals except four obtained between the middle of October and the middle of April weighed between 12 and 19 gm. Three of the four exceptions were parous animals weighing 20–22 gm, and one was a non-parous animal just under 12.0 gm. During the second half of April and May there is a rapid and steady rise in body-weight to the summer level and pregnancies appear simultaneously with its beginning. During July and August there is a steady decrease in the maximum body-weights observed. It may be accounted for, in part by heavy mortality among the larger and presumably older females and partly by a failure of young animals to reach a large body-size in their first season. It is also possible that an actual loss of weight by parous animals occurs.

It is clear that young females born early in the season become mature and breed before its close. *Evotomys* differs in this respect from the Common Shrew which does not breed in the season in which it is born (BRAMBELL, 1935). It is equally obvious that parous animals frequently survive the winter and participate in a second breeding season. It is not possible to decide with certainty, owing to lack of means of distinguishing between parous animals that have bred in one or two seasons, whether any animals survive a second winter and breed in yet a third season but the body-weights would appear to indicate that this is exceptional.

The breeding season begins abruptly in the middle of April, the first pregnancy having been obtained on the 15th but 16 of the 26 animals trapped during the second half of the month were pregnant.

The total number of females examined each month, the number that are adult, and the number pregnant are given in Table III. Only animals with corpora lutea or traces of them in the ovary, with old placental sites in the uterus, or with

TABLE III

Month	Total number	Adults	Pregnant
January	17	1	—
February	33	3	—
March	34	4	—
April	37	28	16
May	82	79	69
June	61	40	38
July	29	24	22
August	57	36	30
September	15	7	3
October	26	6	1
November	14	2	—
December	13	1	—
Total	418	231	179

both, and which therefore have ovulated at some time, are included as adults. Since these structures are so persistent during anoestrus it is improbable that any adult animals have been excluded owing to failure to detect them.

The percentage of adult animals that are pregnant each month, extracted from this table, is represented graphically in fig. 2.

It can be seen that the points fall on a remarkably smooth curve rising steeply from zero in March to a maximum of 95% in June and then declining again to zero in November. It is clear therefore that the breeding season extended, in the years in which the material was obtained, from April to October reaching a peak in

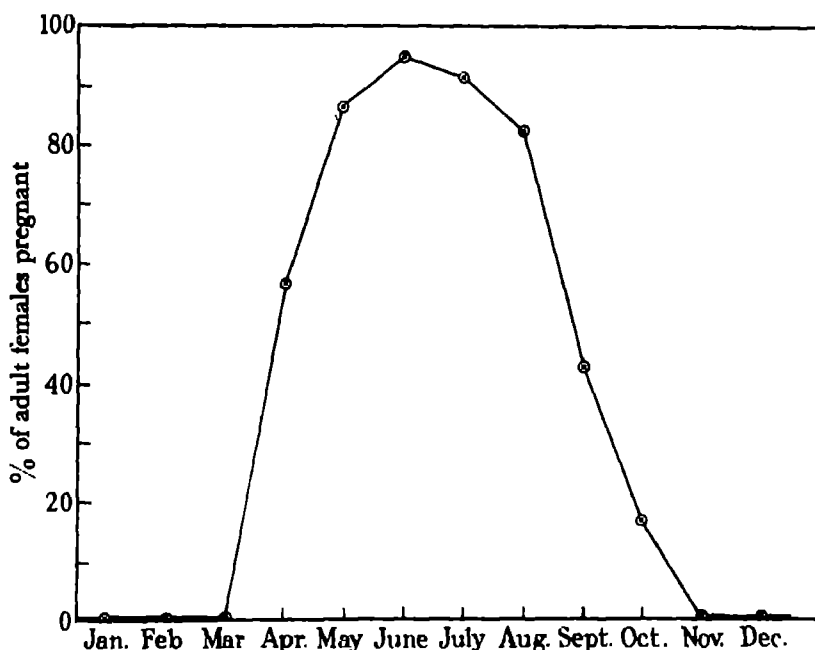


FIG. 2—Graphical representation of percentage of adult animals that are pregnant according to the month. The points are derived from the data in Table III.

June. The latest pregnancy was obtained on October 6. BARRETT-HAMILTON (1911) states that the breeding season in the south of England lasts regularly from March to December inclusive, and may include January and February. BAKER (1930) records that in Oxfordshire the peak of the breeding season is reached in June and that there was complete cessation of breeding, as determined by the occurrence of pregnant females, during the period October to February inclusive in the winters 1925-26 and 1926-27, but not in 1927-28. The results presented in this paper confirm BAKER's statement that breeding reaches a maximum in June. Since most of the winter material was obtained in North Wales in 1931-32 and 1932-33 it is clear that breeding stopped during those years, at least in Caernarvonshire and Anglesey.

Assuming that gestation and suckling together take about six weeks, as in *E. gapperi* (SVIHLA, 1929) or in the white mouse, and that breeding begins in mid-April then young animals of the season might be expected to occur in the traps in the 4th week of May. Actually the first young animal was obtained on May 26, which supports these assumptions.

Examination of fig. 1 leaves little doubt that young animals born at the beginning breed later in the same season. This is borne out by examination of fig. 3 in which the percentages of all females that are adult each month, extracted from Table III, are shown. The first peak is reached in May and is followed by a drop in June,

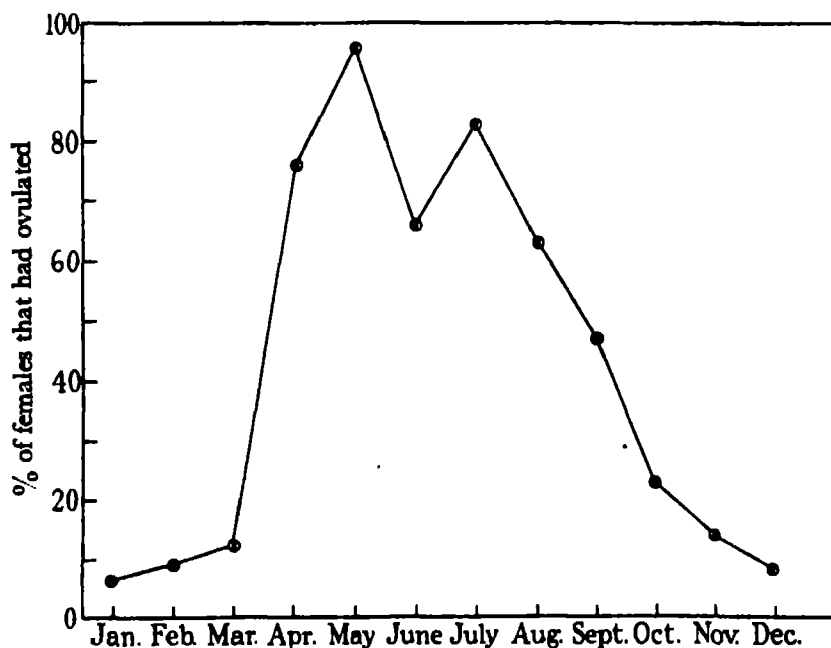


FIG. 3—Graphical representation of the percentage of females that are adult (*i.e.*, have ovulated previously) according to the month. The points are extracted from the data in Table III.

presumably owing to the appearance of immature animals. The second and lesser peak in July is probably due to some of the young animals having become mature.

More direct evidence of breeding in the season in which they were born is provided by the occurrence in the latter part of the season of pregnant animals of light weight which do not appear to be parous.

VI—FERTILITY

It can be seen from fig. 2 that during May, June, July, and August over 80% of all adults are pregnant. Since it may be assumed that the period of gestation is short, as in the white mouse, and since many of the adults during these months are known to be parous it is clear that many animals must be pregnant while suckling

of the breeding season in April. This can be seen from the records for the month (excluding uterine pregnancies) given in Table XIV. The evident short duration of these cycles also suggests that copulation does not occur, since the sterile mated cycle in the white mouse is 12 days, twice as long as the unmated cycle.

TABLE XIV

Date	No. of sets of corpora lutea	Condition
May 4 . . .	None	Non-pregnant
" 5 . . .	"	"
" 7 . . .	"	"
" 7 . . .	"	"
" 12 . . .	"	"
" 12 . . .	"	"
" 13 . . .	3	"
" 13 . . .	2	"
" 14 . . .	3	"
" 14 . . .	2	"
" 18 . . .	2	"
" 19 . . .	3	Pregnant (ova in tubes)
" 22 . . .	4	" "
" 22 . . .	3	Non-pregnant
" 23 . . .	3	"
" 23 . . .	1	"
" 24 . . .	2	"
" 24 . . .	3	Pregnant (ova in tubes)
" 24 . . .	4	Non-pregnant
" 25 . . .	None	"
" 25 . . .	"	1st oestrus with vaginal plug
" 25 . . .	1	Pregnant (ova in tubes)
" 25 . . .	3	" "
" 26 . . .	3	" "
" 26 . . .	3	" "
" 27 . . .	3	" "
" 27 . . .	2	" "
" 27 . . .	3	" "
" 28 . . .	3	" "
" 29 . . .	2	Oestrus with vaginal plug
" 29 . . .	1	Non-pregnant
" 29 . . .	None	"
" 30 . . .	4	Pregnant (ova in tubes)
" 30 . . .	4	" "

b. Oestrus

The primordial follicles in the ovarian cortex measure approximately $15\ \mu$ and the contained oocytes approximately $9\ \mu$ in diameter. The subsequent growth of the ovum relative to the follicle can be divided into two phases, each of which can be

expressed as a straight line regression (BRAMBELL, 1928), as in other mammals. The regression formulae for the two phases are :—

$$(a) \ y = 2.56 + 0.455x, \text{ where } x = 10 \text{ to } 124,$$

$$(b) \ y = 56.84 + 0.018x, \text{ where } x = 125 \text{ to } 800,$$

where y = diameter of oocyte in μ , and x = diameter of the follicle in μ . The fully-grown ovum thus measures approximately 70 μ in diameter. The largest follicle measured 820 μ in diameter but it is probable that there is a good deal of variation in the size of the follicle when it ruptures. At oestrus the follicle appears to reach its full size, fig. 15, Plate 11, about the time that the spindles of the heterotypic division are formed in the oocytes. Six animals with oocytes in this stage were obtained, as shown in Table XV.

TABLE XV

Ref. No.	Stage of oocytes	Mean diameter of mature follicles μ	Mean diameter of corpora lutea of previous oestrus mm	Whether copulation has occurred
E 241	Metaphase of heterotypic division . . .	568	1.15	
E 501	„ „ „ . . .	636	1.10	+
E 548	„ „ „ . . .	686	0.79	+
E 588	„ „ „ . . .	650	1.01	+
E 189	„ „ „ . . .	793	1.01	
E 520	Late anaphase of heterotypic division .	556	None	+

The corpora lutea of the previous oestrus measure 0.8 to 1.2 mm. Copulation occurs at this time, four of the six animals with heterotypic spindles in the oocytes having spermatozoa in the vagina and uterus, while none of those in oestrus, with the large follicles but with vesicular nuclei in the oocytes, contained spermatozoa. One animal, E 241, Table XV, contained one follicle which had just ruptured although the other follicles had not done so. It would appear, therefore, that the heterotypic division is completed after ovulation. Ovulation takes place spontaneously in the absence of copulation.

Three animals with tubal ova containing homotypic spindles were obtained, only two of which had copulated. In one of these, sperm-heads could be distinguished in the cytoplasm of some of the ova. It is apparent, therefore, that fertilization occurs either in the ovarian capsule or in the upper part of the tube and before the separation of the second polar body.

The two animals with ova in the metaphase of the heterotypic division and the other with tubal ova in the homotypic division, which had not copulated support the suggestion (p. 86) that the sterile dioestrous cycles noted, especially at the beginning of the breeding season, are due to failure to copulate rather than to sterile mating.

The condition of the uterus and vagina of *Evotomys* during oestrus closely resembles that of the rat and mouse. The uterus becomes distended with fluid, fig. 16, Plate 11, in a characteristic manner and the vaginal epithelium is intensely cornified, fig. 12, Plate 10. Copulation results in the formation of a hard vaginal plug as in many other small rodents. This plug remains in position for a short time and was found in seven of the ten animals with fertilized but unsegmented tubal ova containing two pronuclei. It was not present, however, in animals with tubal ova undergoing the first cleavage or in the two-cell stage.

c. Pregnancy

The total number of pregnant animals obtained was 179, which were divided between the various stages as in Table XVI.

TABLE XVI

Stage	No. of examples
Tubal stages -	
Ova containing homotypic spindles and sperm-heads	2
Ova with ♂ and ♀ pronuclei	10
Ova with first cleavage spindles	2
2-cell stages	8
2-cell to 4-cell stages	3
4-cell stages	5
4-cell to 8-cell stages	2
8-cell stages	6
16-cell stages	5
Morulae in Fallopian tubes	6
Total	49
Uterine morulae or blastocysts prior to implantation	50
Post-implantation uterine stages	80

The relative frequency of the various tubal stages provides a rough indication of their relative duration. The eggs pass from the Fallopian tube into the uterus as solid morulae in which the primitive yolk-sac cavity appears soon afterwards. Implantation closely resembles that of the mouse and rat. According to KIRKHAM (1916) pregnancy lasted 20 days in the strain of mice he used, the ova were found in the tubes for the first four days; on the fifth day the blastocysts were free in the lumen of the uterus, and implantation then occurred. It is probable that the duration of pregnancy in *Evotomys* is approximately the same as it is in the mouse, but even if it differs considerably, it may still be assumed that the relative duration of the various stages is similar. It follows that a random sample of pregnant animals would tend to the ratio of 4 tubal stages : 1 free uterine stage : 15 post-implantation stages. The observed ratio of 49 : 50 : 80 does not conform to this expectation. The relatively small number of late uterine stages obtained may be due to their

being less easily trapped, since probably they are less active at the end of pregnancy. This explanation can hardly apply to animals with tubal and early uterine stages. If it is assumed that the numbers of these two stages do represent a random sample some other explanation must be sought. To explain a similar ratio observed in trapped material of the Common Shrew, BRAMBELL (1935) suggested that the relatively large number of early uterine stages was due to prolongation of pregnancy in lactating animals. LATASKE (1887) first showed that the period of gestation is considerably prolonged during lactation, and KIRKHAM (1916) demonstrated that this prolongation is due to delay in implantation, the ova remaining free in the uterus without apparently developing further, for a much longer time than in animals which are not suckling. MIRSKAIA and CREW (1931) record the duration of 15 pregnancies in lactating mice which showed a prolongation beyond the normal for non-lactating animals of from 8 to 16 days with a mean of 11·8 days. It is known from the condition of the mammary glands that some of the pregnant animals recorded herein were lactating, and it appears probable, from the high percentage of adults pregnant in June, July, and August, that many were also lactating at the same time.

Since animals in the early part of the season, during April and the first half of May, cannot have been lactating, comparison of the ratio of tubal to early uterine stages obtained with that of those obtained subsequently should show a difference. This can be seen to be so from Table XVII.

TABLE XVII

	Tubal stages	Unimplanted uterine stages
Beginning of season to May 15	23	8
May 16 to end of season	26	42
Total	49	50

It is apparent that the unexpected preponderance of early uterine stages is almost entirely confined to the later part of the season, thus strongly supporting the theory that it is due to delayed implantation in lactating animals. If it is assumed that the delay in implantation bears the same time relation to the period of gestation that it does in the mouse then, on the basis of the data of MIRSKAIA and CREW (1931), the percentage of animals exhibiting delay (*i.e.*, lactating) in the later part of the season can be calculated as in Table XVIII.

TABLE XVIII

Prolongation of pregnancy	% of pregnant animals lactating
Minimum (8 days in mouse)	68
Mean (11·8 days in mouse)	46
Maximum (16 days in mouse)	34

Since some of the pregnant animals will be young non-parous animals and others will be adults which are not suckling owing to accidental loss of the litter it is clear that pregnancy must follow the post-partum oestrus in a large percentage of parous animals and must accompany lactation.

The correspondence of the number of embryos in each of the uterine cornua with the number of corpora lutea in the ovary belonging to it shows that no transference of embryos from one side to the other, such as was observed in the Common Shrew (BRAMBELL, 1935), occurs in this species.

The newly ruptured follicle immediately after ovulation measures approximately

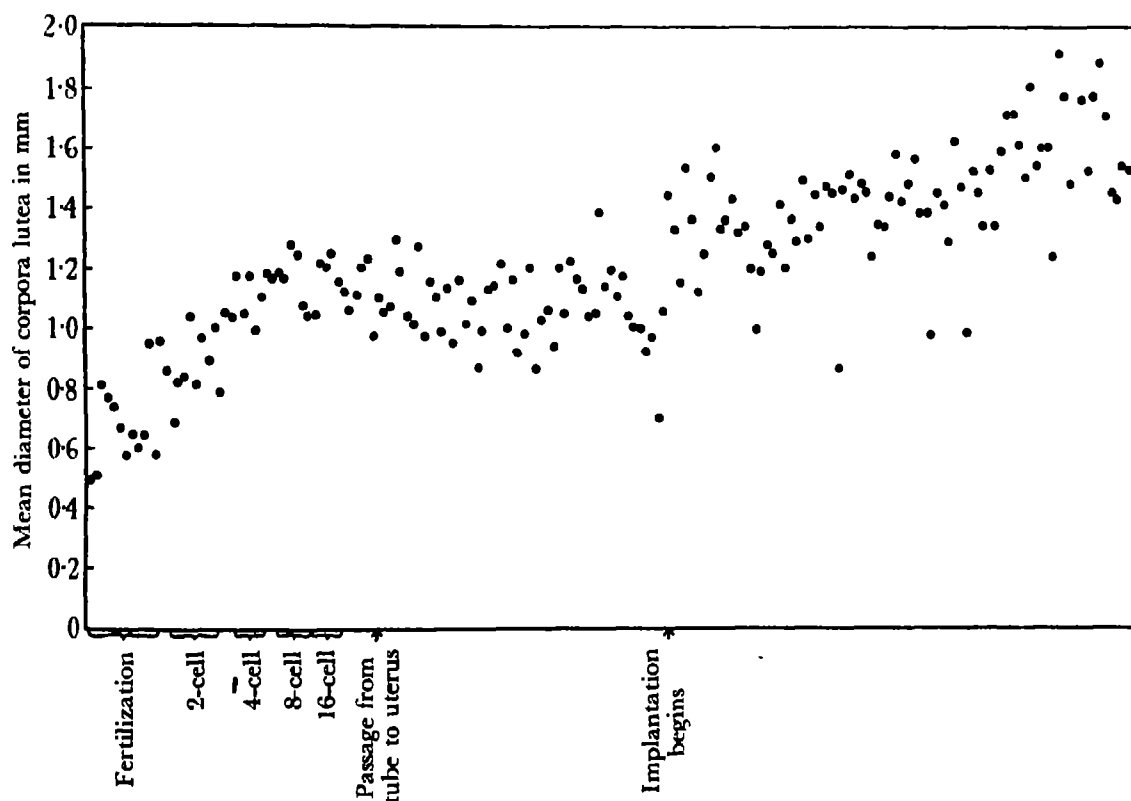


FIG. 9—Graphical representation of the mean diameters of the corpora lutea of pregnancy present in the ovaries of each pregnant animal. The successive stages of pregnancy are arranged in order on the abscissa beginning with fertilization at the origin and ending at approximately full-term on the right. It can be seen that there is an initial period of rapid growth ending, before the ova pass from the Fallopian tube into the uterus, and a second period of growth which begins when implantation occurs and continues until parturition.

0.5 mm in diameter. The subsequent growth of the corpus luteum during pregnancy is represented in fig. 9, in which the mean diameters of the corpora lutea of pregnancy of each animal are plotted. The animals are arranged in order on the abscissa, the earliest stage on the left and the latest on the right. The tubal stages were

seriated with accuracy, microscopically, and the post-implantation stages approximately, by weight. The early uterine stages, prior to implantation, cannot be seriated with any certainty since they develop so little while free in the uterine lumen. It is apparent that the corpus luteum grows rapidly at first, attaining a diameter of 1.0 to 1.3 mm by the time the tubal ova are in the 8-cell stage. Thereafter there is no growth and there may even be a slight decrease in diameter until implantation occurs, when a second less rapid growth phase begins and continues until parturition. It is remarkable that there is no definite decrease in the size of the corpus luteum until after parturition, since in many animals it has been found that retrogression sets in before parturition and is marked by a very rapid decrease in size toward the end of pregnancy.

Since it has been deduced that many of these pregnant animals were lactating, with a prolonged gestation period in consequence, these data provide, for the first time, information as to the growth of the corpus luteum during the prolonged period when the blastocyst is free in the uterine lumen and is consequently in a condition of arrested development. It would be expected, from analogy with the rat and the mouse (p. 89), that without delay due to lactation, free uterine stages should be one-fourth as numerous as tubal stages. Since 49 tubal stages are included it follows that about 38 of the 50, or approximately 75% of early uterine stages, must be accounted for by prolongation of gestation due to lactation. Examination of fig. 9 therefore strongly suggests that, when the development of the blastocysts is arrested and their sojourn free in the uterine lumen is prolonged by lactation, the growth of the corpus luteum is also arrested. This conclusion is of considerable interest. The work of LONG and EVANS (1922) on the rat, and of DEANESLY (1930) on the mouse has shown that after oestrus, accompanied by copulation, the corpus luteum undergoes an initial period of growth which is completed about the sixth or seventh day in the latter species. During this initial period the secretion of the corpus luteum prepares the uterus for implantation, as is shown by the production of placentomata following suitable experimental stimulation of the uterus. Following this initial period, if the copulation was sterile (*i.e.*, was not followed by implantation of the embryos), the corpus luteum regresses; if fertile the corpus luteum enters upon a second growth phase immediately after the time when implantation occurs. It is therefore reasonable to suppose that implantation stimulates directly or indirectly the corpus luteum to enter upon this second growth stage. If this is so, the prolonged resting stage between the two growth phases of the corpus luteum, accompanying the delay in implantation, is easily understood.

Corpora lutea atretica frequently, though not invariably, are formed during pregnancy. They vary in number, as many as six or seven having been found in a pair of ovaries. They do not grow as large as the corpora lutea of pregnancy and they can be distinguished from them by size, as well as by the inclusion of the remains of the ovum and the histological character.

The only pregnant animal (E 968) obtained in October, calls for special consideration. It was 19 gm in weight and was obtained on the 6th. The uterus

contained two unmistakable blastocysts free in the lumen. The condition of the corpora lutea in the ovaries and of the placental sites in the uterus indicate that it had probably become pregnant during a post-lactation oestrus but, since the mammary glands were not preserved, it is just possible that it did so at the post-partum oestrus and that implantation had been postponed by lactation for some days prior to its being trapped. The uterus of this animal, fig. 18, Plate 11, though parous, is exceedingly small and histologically resembles that of an animal in anoestrus, figs. 17 and 19, Plate 11. It is quite unlike the large and active uterus usually associated with uterine blastocysts and in which the mucosa is preparing for implantation. It is almost impossible to believe that the blastocysts could become implanted with the uterus in this condition and it seems more probable that it was entering into anoestrus and that pregnancy would not have been maintained. If this interpretation is correct, then not only can an animal pass into anoestrus during the early stages of pregnancy but the blastocysts can persist in the uterus while it is retrogressing and until it is in an inactive condition characteristic of anoestrus.

Remarkable giant cells were found in the uterus of one animal in which the uterine blastocysts were in process of implantation. The uterine epithelium was disappearing in the immediate vicinity of the blastocysts and they were passing from the uterine lumen into the sub-epithelial tissues. The giant cells, fig. 20, Plate 11, of which there were several, were confined to one cornu and were scattered along its length. They were situated in the mucosa, between the epithelium and the circular muscle and were of enormous size, measuring as much as 0.4 mm in diameter, although the whole cornu was less than 1.0 mm in diameter. They resembled the normal placental giant cells in appearance and probably had a similar origin. In comparison their size was great, even for the placental giant cells at their maximum development, and their occurrence in the uterus prior to the establishment of the placenta is obviously abnormal. It follows that either they have arisen from the uterine tissues or else, if of embryonic origin, they have persisted from a previous pregnancy. The condition of the uterus indicated that the animal was probably non-parous so that the latter alternative is improbable. The former alternative is in accord with the views of many (*see* SANSOM, 1927), but not all, embryologists who have investigated the origin of the placental giant cells in rodents.

The changes in the reproductive organs typical of pregnancy closely resemble those of the mouse. The uterine and vaginal changes and the development of the mammary glands are similar. At the end of pregnancy the vaginal epithelium becomes greatly thickened and undergoes intense mucification, fig. 13, Plate 10, and fig. 21, Plate 11, as in the mouse and the rat.

d. Lactation Anoestrus

There is an immediate post-partum oestrus in *Evotomys*, as in many other animals. This oestrus is easily identified by the condition of the uterus and by vaginal

cornification as in the mouse. The material provides no evidence of oestrus occurring during lactation in animals which do not become pregnant at the post-partum oestrus. The inactive condition of the reproductive organs in non-pregnant lactating animals indicates that there is a lactation anoestrus which probably extends throughout lactation. There is probably a post-lactation oestrus but at the end of the season some animals may pass directly from lactation to winter anoestrus. The condition of the corpora lutea and mammary glands of some early pregnancies suggests that they became pregnant at a post-lactation oestrus period (*see* E 968, p. 92). Two oestrous animals, obtained in June and August respectively, had only very old corpora lutea in the ovaries. The oestrous period, therefore, could not have followed a dioestrous cycle and the condition of the uterus shows that it was not post-partum. It must therefore have followed a period of anoestrus, presumably lactation anoestrus, since both were obtained during the height of the breeding season. The uteri, however, contained no visible placental sites so that, if the animals had just finished lactating, these must have retrogressed much more rapidly than they do during winter anoestrus.

e. Winter Anoestrus

Animals in winter anoestrus are easily identified by the atrophy of the reproductive organs and their inactive appearance on histological examination. The ovaries contain neither large follicles nor recent corpora lutea. Throughout the greater part of the winter the ovaries, in mature animals, contain small fibrosed remnants of the old corpora lutea, and even in the spring, just before the beginning of the breeding season, patches of pigment in the stroma indicate their former presence. The uteri are very small, *figs.* 17 and 19, Plate 11, but the remnants of placental sites in parous animals persist in the base of the mesometrium throughout the winter. The vaginal epithelium is thin and inactive, *fig.* 14, Plate 10, and the lumen is reduced in size. The vaginal orifice during anoestrus becomes closed by epithelial fusion. This does not occur in the shrew (BRAMBELL, 1935), which has a single urino-genital orifice in the female, but is possible in *Evotomys*, in which the urethra opens on the clitoris and is thus entirely separate from the vagina.

The authors' thanks are due to Dr. A. S. PARKES, F.R.S., and to Mrs. PARKES for their advice and for collecting part of the material from the Home Counties, to Professor J. P. HILL, F.R.S., for advice, and to Messrs. L. H. JACKSON, H. A. COLE, and W. H. EDWARDS for much assistance in collecting the material.

The authors are indebted to the Rt. Hon. Lord PENRHYN for permission to trap on his estates, where many of the animals were obtained.

The expenses of this research were defrayed in part by grants from the Government Grant Committee of the Royal Society to one of us (F. W. R. B.), for which we wish to express our thanks.

VIII—SUMMARY

The material consisted of 1036 Bank Voles obtained between March, 1931, and May, 1933, chiefly in North Wales, of which 443 were females.

The sex-ratio of the whole of the material trapped was 57.24 ± 1.04 males per cent.

The anatomy of the reproductive organs is similar to that of the mouse. There are closed ovarian capsules and separate cervical canals. The urethral orifice is distinct from that of the vagina. There are two pairs of thoracic and two pairs of abdominal mammae.

The weight of the young at birth is estimated at 1.5 to 2.5 gm. The lightest animal trapped was 6.4 gm, and the heaviest 33.8 gm. The lightest parous or pregnant animal was 12.5 gm. During the winter the weights range from 12 to 19 gm with few exceptions. The body-weights rise rapidly during the second half of April and May.

Females born early in the breeding season breed before its close. Such parous animals frequently survive the winter and breed again the following year.

The breeding season extends from the middle of April until the beginning of October and reaches a maximum in June.

It is estimated that one female may rear four or even five litters in a season.

The mean number of ova ovulated at each oestrus is 4.43 as estimated from a sample of 277 sets of corpora lutea; the largest number observed was 12. The corpora lutea are distributed at random between the two ovaries of a pair. It is deduced that the number of ova ovulated at each oestrus is not limited by the capacity of the ovaries to produce a sufficient number of mature follicles.

The mean number of foetuses *in utero* in 70 late pregnancies was 4.11 and the largest number observed was 6. Similarly the mean number of placental sites in 58 pairs of parous uteri was 4.48 and the largest was 7.

The mean number of ova ovulated at each oestrus rises during April and May to a maximum in June and falls off thereafter. It is also directly related to the body-weight. This relation is expressed as a straight line regression on body-weight. The seasonal variation and the relation to body-weight cannot be considered independent but the data do not permit a decision as to which is the more fundamental. The mean number of foetuses *in utero* in late pregnancies also varies seasonally and with body-weight.

The incidence of intra-uterine mortality is much heavier when 6 or more, than it is when 5 or less, ova are ovulated. It is concluded that this increased mortality is due to deficiency of materials required for development and not to mechanical overcrowding of the uterus.

At the beginning of the breeding season the majority of females go through a variable number of dioestrous cycles, most frequently 3 but sometimes more, before becoming pregnant. A few become pregnant at the first oestrus. These sterile cycles are probably not accompanied by copulation. Since the males at this time are in full breeding condition the failure to copulate must be due to the females.

Animals attaining puberty during the latter part of the breeding season become pregnant at the first oestrus much more frequently.

The growth of the ovum and follicle is described. The follicles have a mean diameter of 550 to 800 μ at the time of ovulation. The heterotypic spindle is formed before ovulation and the division completed subsequently. Copulation normally occurs shortly before ovulation but after the formation of the heterotypic spindles in the ova. Ovulation occurs spontaneously in the absence of copulation. The spermatozoon enters the ovum before the completion of the homotypic division and the separation of the second polar body. During oestrus the uterus is distended with fluid and the vaginal epithelium is intensely cornified, as in the mouse. Copulation results in the formation of a hard vaginal plug which remains in position until the ova are in the stage with two pro-nuclei.

The total number of pregnant animals obtained was 179, of which 49 had tubal ova, 50 uterine blastocysts free in the lumen and 80 implanted embryos. The ovum passes from the Fallopian tube into the uterus as a morula.

There is evidence that many parous animals are pregnant and lactating simultaneously and that, in these, pregnancy is prolonged by lactation causing a delay in implantation. This delay results in the blastocysts remaining in a resting stage in the uterine lumen for a considerable period.

The newly ruptured follicle measures 0.5 mm in diameter. During pregnancy the corpus luteum grows rapidly until the tubal ova are in the 8-cell stage approximately, when it is 1.0 to 1.3 mm in diameter. It remains at this size until implantation occurs, even when this is delayed by lactation, and then enters upon a second growth phase which continues until the end of gestation.

One animal obtained in October had blastocysts free in the uterine lumen but the uterus was atrophied and in the anoestrous condition.

At the end of gestation the vaginal epithelium becomes very thick and undergoes intense mucification as in the mouse.

There is an immediately post-partum oestrous period, and after lactation animals probably come into oestrus again if they did not become pregnant at the post-partum oestrus.

Non-pregnant lactating animals exhibit a lactation anoestrus.

There is a well-marked winter anoestrus during which the vaginal orifice is closed.

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X—DESCRIPTION OF PLATES

PLATE 10

FIG. 10—Photograph of the ventral aspect of the adult female reproductive organs dissected so as to show them *in situ* in their natural relations. The pubic symphysis has been removed to expose the vagina. $\times 1.9$.

An outline key is provided in fig. 10A.

Abbreviations :—*b*, bladder ; *c*, median portion of uterus containing the separate cervical canals of the two uterine cornua ; *cl*, clitoris ; *f*, Fallopian tubes ; *k*, kidney ; *m*, mesometrium ; *o*, ovaries ; *p*, prepuce glands of clitoris ; *r*, rectum ; *s*, suprarenal glands ; *u*, uterine cornu ; *w*, ureter ; *v*, vagina ; *vo*, vaginal orifice.

FIG. 11—Photomicrograph of a section of the ovary of an adult (E 570), obtained in May with tubal ova, showing four generations of corpora lutea. The newest are numbered 1 and the oldest 4. $\times 32$.

FIG. 12—Photomicrograph of a section of the vaginal epithelium during the first oestrus (E 520). A vaginal plug was present and the mature follicles contained oocytes in anaphase of the heterotypic division. The epithelium shows intense cornification. $\times 370$.

FIG. 13—Photomicrograph of a section of the vaginal epithelium at the end of pregnancy (E 680). The epithelium is undergoing mucification. A transverse section of the same vagina is shown at a lower magnification in fig. 21, Plate 11. $\times 370$.

FIG. 14—Photomicrograph of a section of the vaginal epithelium of a parous animal (E 1045) during winter anoestrus. The debris in the uterine lumen in the lower part of the figure contains nucleated epithelial cells and leucocytes. The epithelium is very thin. $\times 370$.



FIG. 11

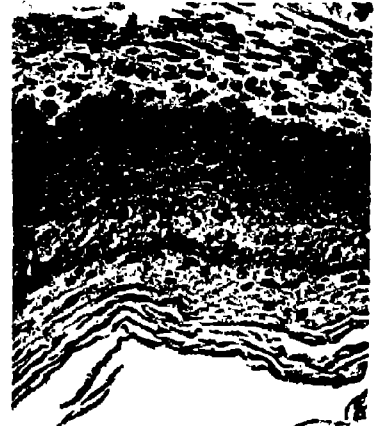


FIG. 12.

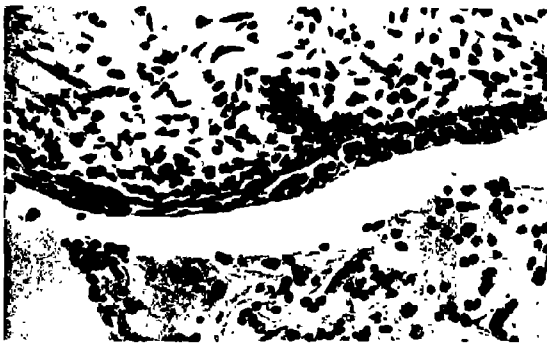


FIG. 14.

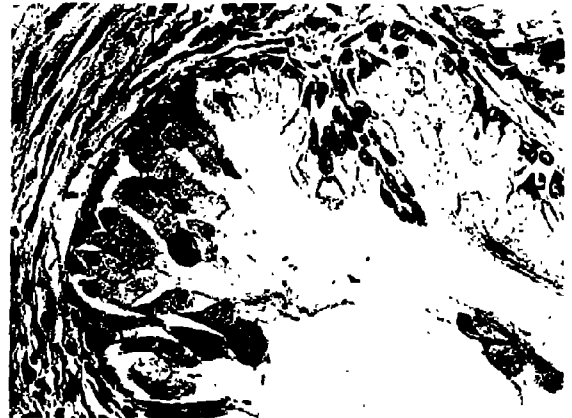


FIG. 13



FIG. 10.

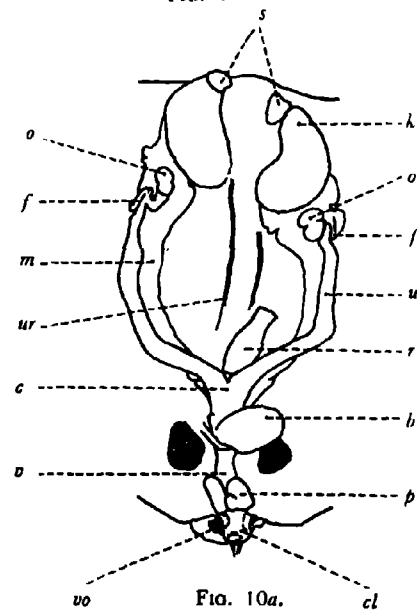


FIG. 10a.

PLATE 11

- FIG. 15—Photomicrograph of a transverse section of a mature follicle about to ovulate (E 189). The oocyte was in metaphase of the heterotypic division although the chromosomes do not appear in this section. $\times 87$.
- FIG. 16—Photomicrograph of a transverse section of the uterine cornu during oestrus (E 501). The extreme distension of the uterus with fluid and the consequent thinning of the uterine wall are apparent. $\times 24$.
- FIG. 17—Photomicrograph of a transverse section of the uterine cornu of a non-parous animal (E 402) during winter anoestrus. $\times 120$.
- FIG. 18—Photomicrograph of a transverse section of the uterine cornu of a parous animal with unimplanted uterine blastocysts obtained in October (E 968). Comparison with figs. 17 and 19 shows that the uterus is passing into the anoestrous condition and that implantation presumably would not take place. $\times 120$.
- FIG. 19—Photomicrograph of a transverse section of the uterine cornu of a parous animal (E 419) during winter anoestrus. Comparison with fig. 17 shows the difference in size and structure of parous and non-parous uteri during anoestrus. $\times 120$.
- FIG. 20—Photomicrograph of a transverse section of the uterine cornu of a pregnant animal (E 603), in which the uterine blastocysts were becoming implanted, showing two abnormal giant cells in the mucosa. $\times 75$.
- FIG. 21—Photomicrograph of a transverse section of the vagina at the end of pregnancy showing mucification of the epithelium. Part of the epithelium is shown at a higher magnification in fig. 13, Plate 10. $\times 22$.

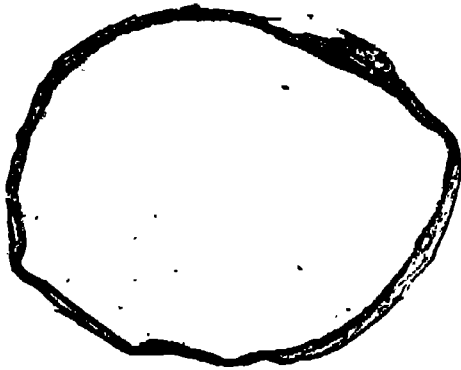


FIG. 16.



FIG. 15.



FIG. 17.



FIG. 19.

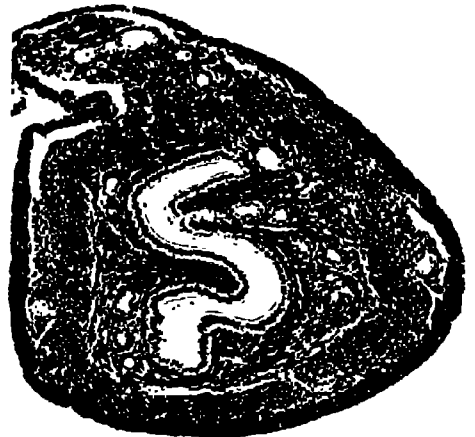


FIG. 18.



FIG. 21.

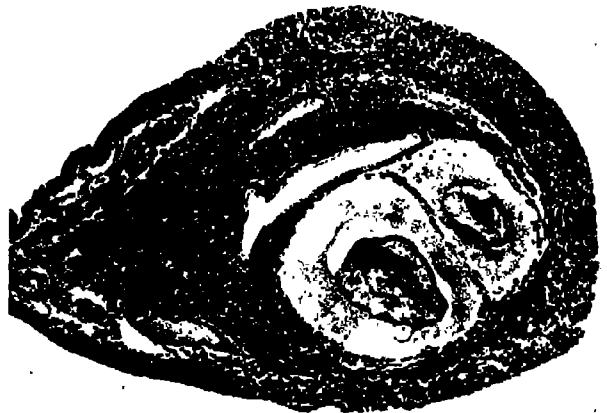


FIG. 20.

Reproduction of the Bank Vole (*Evotomys glareolus*, SCHREBER)*

II—Seasonal Changes in the Reproductive Organs of the Male

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(Communicated by A. S. PARKES, F.R.S. Received July 26, Read November 14, 1935)

[PLATES 12–15]

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I—INTRODUCTION

The present paper deals with the male *Evotomys* and is concerned with two main problems; the duration of the breeding and the non-breeding seasons, and the structure and growth of the reproductive organs. It is, in consequence, complementary to the preceding paper on the female. The only previous work we are aware of on the male of the British species is that of BAKER (1930) on a series of 359 males trapped near Oxford over a period of three consecutive years. BAKER,

* Since this paper went to press the British Museum have published a "List of British Vertebrates," in which the generic name "*Clethrionomys*" is used instead of "*Evotomys*."

taking the arbitrary criterion of 14 gm body-weight or over, found that 260 of these were adult. If spermatozoa were abundant in the tail of the epididymis, as determined from teased preparations, and also if the seminal vesicles were found to weigh 100 mg or over, the animal was said to be fecund. BAKER found that none of the 13 adult males obtained during the winter, October to February inclusive, of 1925-26 was fecund, while 16% of the 45 obtained in the following winter and 52% of the 27 obtained in the winter of 1927-28 were fecund. Thus he concluded that in one of the three winters concerned there was complete sterility.

II—MATERIAL AND TECHNIQUE

The total material consists of the 593 males obtained over the same period and in the same way as the females described in Part I. The body-weight was not known in four and the weights of the reproductive organs in six others. Thus 583 males were available for all purposes involving weights of body and reproductive organs.

The body-weights (± 0.25 gm) were determined, and the reproductive organs were dissected out and removed in one piece. The organs were fixed whole in the alcoholic modification of Bouin's fluid. This fixative was chosen because of its rapid penetration and uniformity of action. The organs were transferred to 70% alcohol and stored for subsequent weighing. The testes, epididymides, seminal vesicles and prostate together, Cowper's glands, prepuce glands, and the penis were weighed on a torsion balance, accurate to 0.5 mg, after the superficial moisture had been removed on a pad of muslin.

The testes of all animals obtained from July to March inclusive, of all with testes under 500 mg, and a sample of 17 of the 199 adults with testes over 500 mg from April to June inclusive were sectioned. The sections were stained with Mayer's haemalum and aqueous eosin. The presence of mature spermatozoa in the testis, determined histologically, was considered a criterion of fecundity, whether they were few in number or numerous. Spermatozoa which retained the cytoplasm of the spermatid were not considered to be mature for this purpose.

During June, 9 animals had testes weighing under 100 mg and all these were devoid of spermatozoa. Otherwise all obtained during April, May, and June had testes over 100 mg. Testes weighing between 100 and 500 mg, numbering 41, were sectioned and found to contain spermatozoa. The presence of spermatozoa was determined histologically in 17 of the animals with testes over 500 mg, and as all gave positive results the remaining 182 were assumed to contain spermatozoa.

The mean diameter of the spermatic tubules in a testis was obtained as follows :—outline drawings were made, by means of a vertical projection apparatus, at a known magnification, of a group of 20 tubules situated at the periphery of a major transverse section of the testis. The greatest width at right angles to the long axis of the section of each tubule was taken as the diameter and the mean of the group of 20 was calculated.

The mean area of the interstitial cells was derived from camera lucida drawings of six to eight cells made at a magnification of 1000 diameters.

The data included in the present paper are given either in the form of scatter diagrams or as correlation tables. The latter were employed where the scatter of points was too close to permit of scatter diagrams being made, except on a large scale. No attempt was made to treat the data statistically in most cases, as it was felt that the relevant information was obvious from the diagrams and tables. Mathematical treatment was employed, however, for the spermatid tubules and interstitial cells. The diameters of the former were plotted against the cube root, and the areas of the latter against the square root of the weight of the testes. This method was adopted in order to express the size of the testes in comparable terms; linear and plane respectively. In both cases a straight regression line was fitted and its significance tested, by using FISHER's (1930) table of *t*.

III—STRUCTURE OF THE REPRODUCTIVE ORGANS

The general arrangement of the reproductive organs of the male *Evotomys* resembles that of the mouse, but differs in details of the anatomy of the accessory glands.

The vasa deferentia are not provided with any visible ampullae, but each is surrounded at its base close to its junction with the urethra by a collar composed of several little lobed glands, fig. 9, Plate 13. These are normally hidden by the prostate, seminal vesicles and bladder.

The seminal vesicles in the adult during the breeding season are large sacculated organs, fig. 7, Plate 12, similar to those of the mouse, but shorter, thicker, and more regular in outline.

The prostate is composed of four pairs of lobes, figs. 7 and 8, Plate 12, surrounding the base of the bladder. Each lobe is composed of a number of unbranched tubules often bent upon themselves and joined together by connective tissue. One pair is situated ventrally, lying on the neck of the bladder immediately anterior to the pubic symphysis, fig. 8, Plate 12. These join the ventral surface of the prostatic portion of the urethra on either side close to the middle line where it joins the bladder. A second and larger pair overlaps these and the ventro-lateral surfaces of the urethra where the vasa deferentia join it. Two pairs of lobes, fig. 7, Plate 12, are situated on the dorsal surface of the urethra covering the bases of the seminal vesicles. The outer of these are long and narrow and lie along the inner sides of the curves of the seminal vesicles on each side, to which they are loosely attached. The inner pair is shorter and wider and meets in the mid-dorsal line. Both pairs join the urethra immediately behind the bases of the seminal vesicles.

The bulbus urethrae, fig. 9, Plate 13, is prominent and is situated at the base of the penis between the crus penis and the anus. The bulbo-cavernosus muscles, which are striped, surround the bulbus on all sides, except dorsally where the muscles

do not extend between it and the rectum. A band of bulbo-urethral muscle, however, extends dorsally above the rectum. The glands of Cowper (bulbo-urethral glands) are pyriform in shape and are situated dorso-laterally to the bulbus and close to its anterior margin, figs. 7 and 9, Plates 12 and 13. They are thus situated on each side of the rectum close to the posterior margins of the ischia. The glands themselves are outside the bulbo-urethral muscles but their ducts run through these to open into the beginning of the cavernous part of the urethra. A large urethral sinus, fig. 7, Plate 12, occupies the centre of the bulbus. It is bilobed with glandular walls and a large central cavity which is continuous and which opens below directly into the urethra, fig. 1, close to the openings of the ducts of Cowper's glands. The

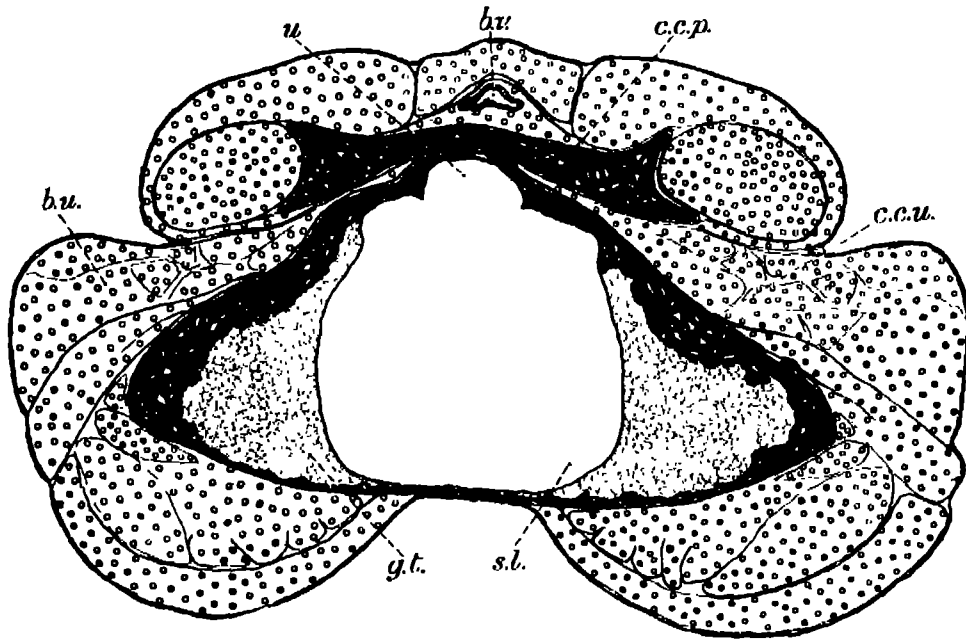


FIG. 1—Diagrammatic transverse section of the bulbus. The bulbo-urethral muscle, *b.u.*, on each side of the urethra, surrounds the urethral sinus, which consists of glandular tissue, *g.t.*, and erectile tissue of the corpora cavernosa urethrae, *c.c.u.* The lumen of the sinus, *s.l.*, is large and is seen to open into the urethra, *u.* *c.c.p.* = corpus cavernosum penis. *b.v.* = blood vessel.

erectile tissue of the corpus cavernosum urethrae extends around this sinus, being especially well developed laterally. The urethral sinus was recognized first by TULLBERG (1899, his Plates XLVIII-LI) in certain rodents and was found in the mouse by GROSZ (1905), and in other species by RAUTHER (1909). Since its structure and development are being investigated at present in this laboratory it is unnecessary to describe the sinus further here. Possibly it corresponds to the "bulbar glands" of MOSSMAN, LAWLAH, and BRADLEY (1932) who recently described them in a number of species of Sciuridae. There is no duct comparable to the long "penile duct" of many Sciurids, described by these authors, since the urethral sinus in *Eutamias* opens directly into the urethra.

The prepuccial glands, fig. 9, Plate 13, are very flattened leaf-like glands lying on each side of the prepuce and closely attached to the superficial fascia of the ventral abdominal wall. They open by means of short ducts into the prepuccial sac. During the non-breeding season these glands are very minute.

IV—GROWTH AND BREEDING SEASON

The body-weights of 589 males were available and are given in the form of a scatter diagram against month in fig. 2. During January and February the body-weights, with one exception at 19 gm, are between 12.5 and 17.5 gm. In March and April they rise rapidly, attaining a mean weight of 23.8 gm during May, when the breeding season is at its height. The heaviest animal was 29.9 gm and was obtained in May. During June, July, August, September, and the beginning of October the downward spread of body-weights is much greater, owing to the presence of young animals from 8 gm upwards. Only one animal under 8 gm was trapped. The maximum observed in each of the five months respectively being 29.7, 26.8, 25.5, 24.2, and 22.2 gm. By the months of November and December the body-weights have attained the winter range of 12.4 to 18.4 gm, with two exceptionally heavy animals in December at 20 and 22 gm respectively.

Fecundity, as judged by the presence of mature spermatozoa in the testes, is attained in March, as will be shown subsequently. The body-weight at which fecundity is attained is not, however, sharply defined. The lightest animal with spermatozoa in the testis was 13.5 gm and the heaviest without spermatozoa was 18.5 gm. A line drawn from 18 gm on March 1 to 15.5 gm on March 31 would, however, separate the fecund above from the infertile animals below, with few exceptions. During June, July, and August the correlation of fecundity with body-weight is even less clearly defined, the lightest animal with spermatozoa being 11 gm and the heaviest without spermatozoa being 17 gm. The numbers for each gram of body-weight are given in Table I.

TABLE I—(JUNE, JULY, AND AUGUST)

Body-weight gm	With spermatozoa	Without spermatozoa
18<	45	—
17 — 17.9	10	1
16 — 16.9	15	5
15 — 15.9	12	4
14 — 14.9	4	7
13 — 13.9	7	5
12 — 12.9	2	4
11 — 11.9	3	8
> 10.9	—	5

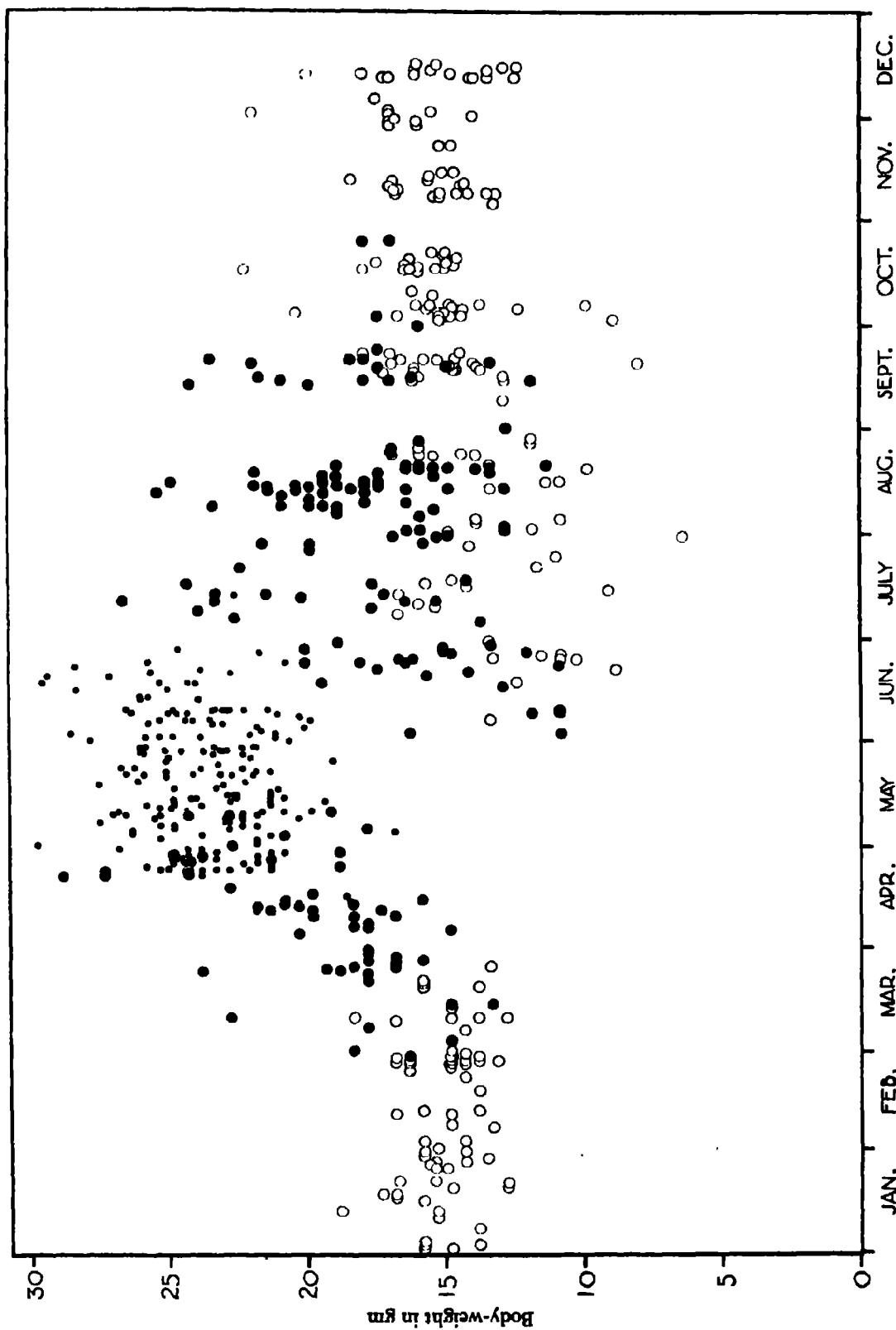


FIG. 2.—Distribution of body-weights throughout the year. The large hollow circles represent animals in which spermatozoa are absent from the testis. The large solid circles denote the presence of spermatozoa in the testis while the presence of spermatozoa is assumed in animals represented by the small solid circles.

It is apparent that the line above which 50% or over are fecund occurs at approximately 14 gm body-weight, and this may be taken as the weight at the time of puberty. It is clear in consequence that those young animals which become sexually mature in the summer of their first season may do so at a lighter body-weight than that which animals reaching puberty in the following spring have attained.

It will be shown subsequently, from histological examination of the testes, that the winter animals include some which have previously been mature, and are consequently in a state of quiescence, as well as young animals, presumably born late in the season, which have not attained puberty. It is clear, therefore, from the distribution of body-weights in late summer and autumn that the heavier adult animals, presumably born during the previous season, either die or undergo a loss of body-weight during the autumn. The ranges of body-weight of the pre- and of the post-pubertal animals overlap during the winter months.

The extent of the breeding season in the male *Evotomys* may be judged by the occurrence of mature spermatozoa in the testes, fig. 16, Plate 15. Using this criterion it is apparent that the breeding season extends from March to September inclusive. Outside this period only five animals with spermatozoa were caught, one on February 28 and four in October. The data are given in Table II and the percentage of animals with spermatozoa in the testes is represented graphically in fig. 3.

It can be seen that none occurs in November, December, and January. The peak of the breeding season is reached in April and May when 100% have spermatozoa,

TABLE II—NUMBER OF ANIMALS WITH AND WITHOUT SPERMATOZOA OBTAINED EACH MONTH

Month	Total number	Without spermatozoa	With spermatozoa	Spermatozoa presumed	% with spermatozoa
January	28	28	—	—	0·0
February	29	28	1	—	3·4
March	33	13	20	—	61·0
April	60	—	30	30	100·0
May	103	—	7	96	100·0
June	86	9	21	56	89·5
July	35	12	22	1	65·7
August	77	19	58	—	75·3
September	41	24	17	—	41·5
October	45	41	4	—	8·9
November	26	26	—	—	0·0
December	24	24	—	—	0·0
	587				

but a second smaller peak occurs in August. This must mean that animals born in the earlier part of the year reach maturity in the same season, but the condition of the winter testes shows that other animals, probably born at

the end of the season, do not reach maturity until the following spring. The testes, fig. 12, Plate 14, of one or two animals obtained at the beginning of the period of rapid hypertrophy, which precedes the onset of the breeding season, show clearly that they have been mature in the preceding season. This demonstrates that mature animals can pass through the non-breeding season in a state of quiescence and become sexually active again the following year.

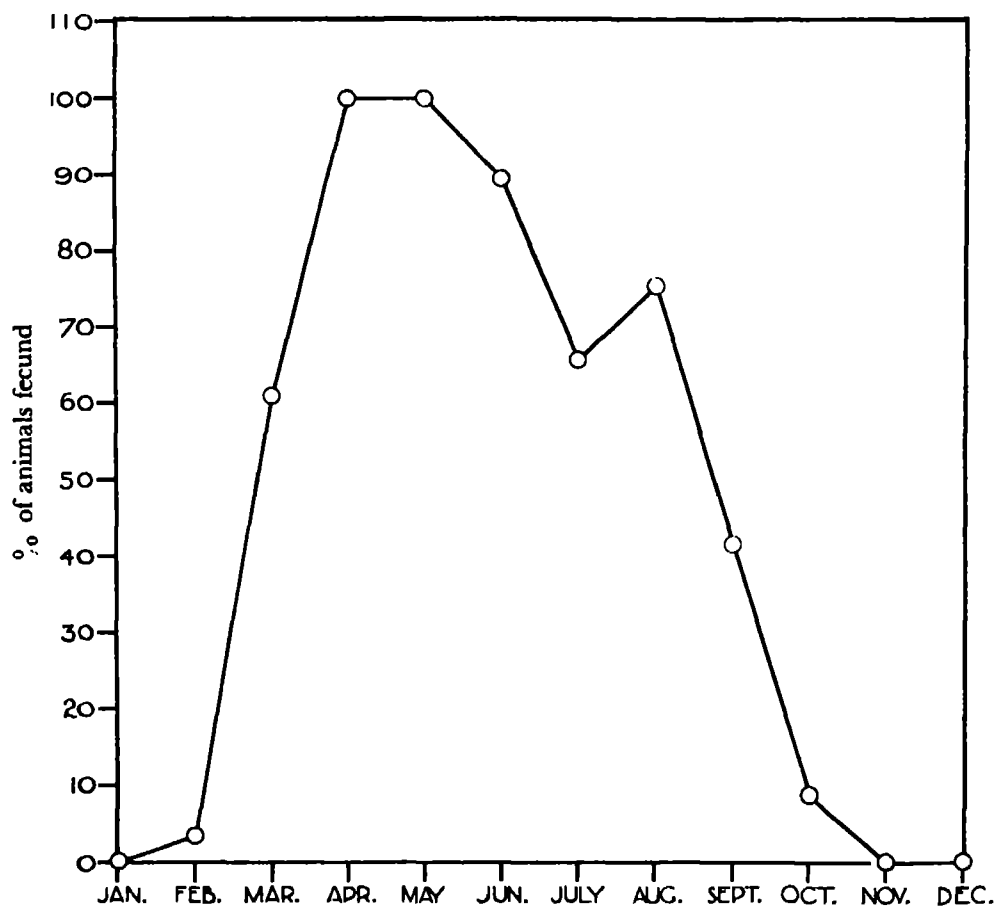


FIG. 3—Graph giving monthly percentage of animals with spermatozoa in the testis. During the months of April, May, and June the presence of spermatozoa is assumed in some of the animals with large testes.

V—TESTES

The combined weights of the two testes of 587 animals are given in the form of a scatter diagram in fig. 4. In a few animals only one testis was available; when this occurred twice its weight was taken. During the winter months of November, December, January, and the first three weeks of February the testes weigh from 3.5 to 40 mg, the average during the first three months being 10.8 mg for all animals, and 23.7 mg for the infertile adults alone. The testes of the immature

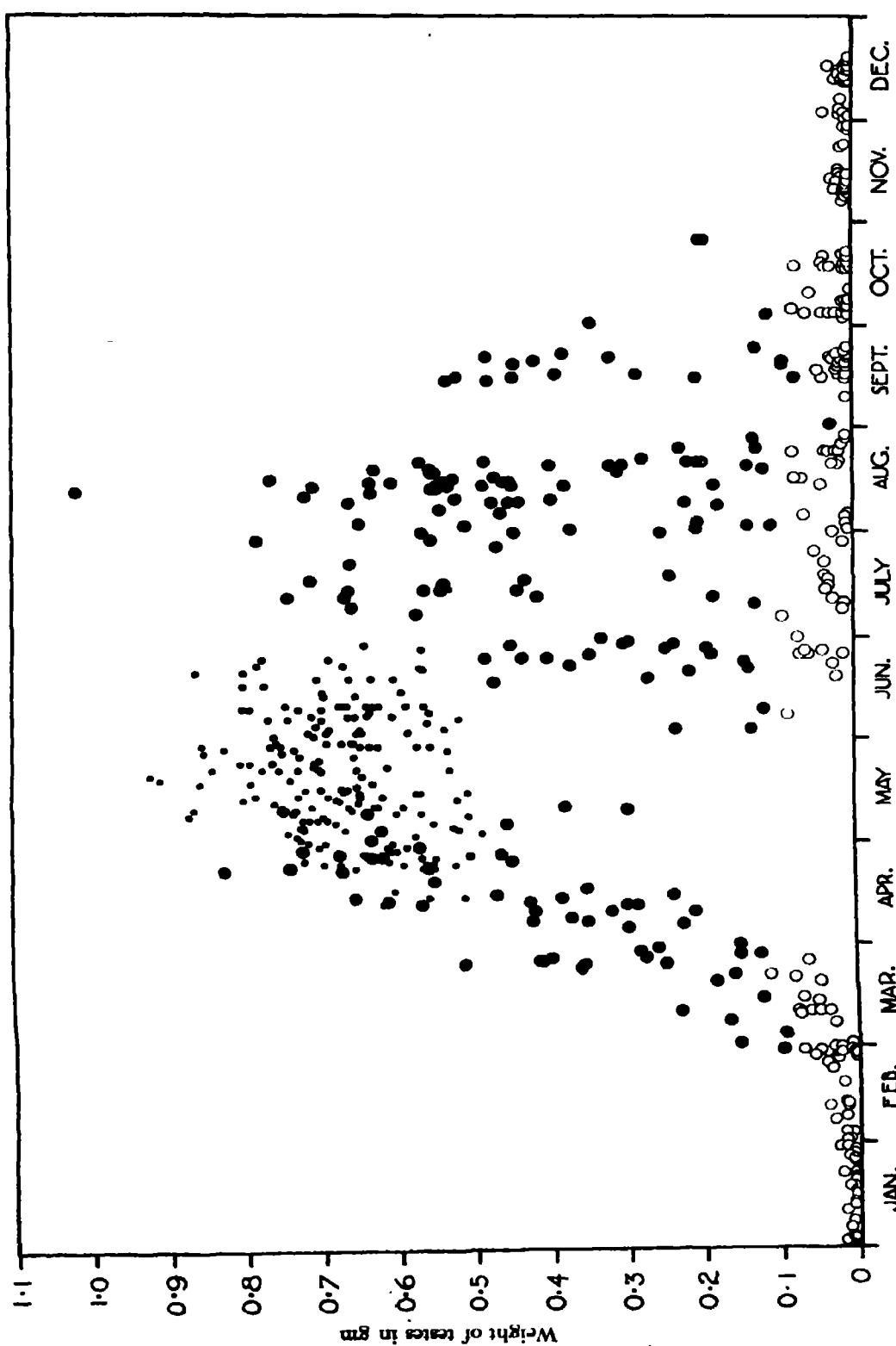


Fig. 4—Distribution of the weights of the testes throughout the year. The presence (actual and assumed) and absence of spermatozoa in the testes is denoted similarly to fig. 2.

animals during these months are generally somewhat lighter than those of the adults. They begin to increase in weight during February and do so rapidly during March and April, attaining a mean of 682 mg in May. The variation in the weights of the testes during the height of the breeding season is, however, considerable, the highest weight recorded in May being 922 mg and the lowest 298 mg. Subsequently the highest recorded weights fall off regularly, with the exception of a single animal in August in which the testes were 1.02 gm, the heaviest recorded. The maximum for each month, excepting this animal, is : June 865 mg, July 782 mg, August 765 mg, September 535 mg, and October 343 mg. The downward spread after May merges with the upward spread of the young animals. The most remarkable conclusion is that the testes of adult animals average 682 mg in May but only 24 mg during the winter months.

The presence of mature spermatozoa is correlated remarkably closely with the weight of the testes. All those at 100 mg and over, which were examined, were found to contain spermatozoa except one pair in March of 113 mg. No testes less than 100 mg in weight contained spermatozoa, with the exception of five animals. Of these, one was in March with testes weighing 96 mg, while the remaining four were in September, with testes at 30, 77, 91, and 92 mg respectively, and were probably animals which had bred and were in a state of regression, figs. 17 and 18, Plate 15, the spermatozoa having remained in the tubules after the testes had dropped below the critical size. The close correlation between the presence of spermatozoa and testes weighing 100 mg and over is in marked contrast with the absence of any such relation between body-weight and fertility.

The relation of the weight of the testes to the body-weight is clearly brought out in Table III in which the data for 583 animals are given in the form of a correlation table. Whereas the correlation is obviously significant and approximates to a straight line relationship the spread of the data is wide, especially for the lower testis weights, as would be expected from the foregoing results.

It is not the purpose of this paper to discuss the detailed histology of the testis except as regards the bearing of this on the problems of the duration of the breeding and non-breeding seasons. No attempt is made therefore to describe the development of the testis or its condition during the breeding season.

The histology of the testis during the winter months has, however, a very direct bearing on the problems with which this paper is concerned. During November, December, and January all the testes exhibit an entire cessation of spermatogenesis and the tubules are completely inactive. They are small in size and generally devoid of a lumen. Within the tubules Sertoli nuclei are numerous but the only germ-cells present are spermatogonia. The spermatogonia are situated next the wall of the tubule and between it and the zone of Sertoli nuclei surrounding the centre of the tubule, which is filled with the cytoplasm of the cells and is free from nuclei. The nuclei of the spermatogonia are large, spherical, and stain less densely than the small oval Sertoli nuclei, from which they are easily distinguished. The chromatin in the resting spermatogonial nucleus is very finely granular and uniformly

dispersed, giving the nucleus its characteristic appearance. One, two or more conspicuous plasmosomes are present close beneath the nuclear membrane.

The testes of all animals obtained during the months of November, December, and January in the winters of both 1931 and 1932, resembled each other in these respects but otherwise they fell into two clearly defined groups.

The testes of the first group, figs. 13 and 15, Plates 14 and 15, are characterized by their flabby appearance on dissection and in sections by irregularity in outline arising from crumpling of the tunica albuginea. The tunica albuginea itself is very thick, has a strong affinity for eosin and its surface is traversed by fine folds as though it had undergone considerable contraction. Within the tunica the tubules are loosely arranged with relatively enormous inter-tubular spaces between them. The tubules themselves exhibit a characteristic wide zone of faintly eosinophil and rather fibrillar cytoplasm next the wall of the tubule which is free from nuclei. This zone separates the Sertoli nuclei, which form a ring around the centre of the tubule, from the wall. The spermatogonia are not plentiful and are situated in this cytoplasmic zone close to the Sertoli nuclei rather than close to the wall. Some of the tubules have a distinct lumen but in others it is occluded.

The second group, figs. 10 and 11, Plates 13 and 14, differs strikingly from the first, in that the testes appear firm on dissection and are plump and regular in contour. The tunica albuginea in sections is thin and evenly extended over the surface of the testis. Within, the spermatatic tubules are closely packed together, the interstices being occupied by interstitial tissue and lymphatic spaces. The tubules themselves differ from those of the other group of animals in that the zone of Sertoli nuclei is situated close to the wall of the tubule, without a wide intermediate zone of cytoplasm. Spermatogonia are much more numerous and there is no trace of a lumen in the tubules. These testes closely resemble those of young animals in summer prior to the onset of puberty and it would appear reasonable to assume that they are in fact prepubertal. The testes of the first type, on the other hand, obviously belong to animals which have bred previously and which are therefore in true non-breeding condition. The characteristics of these testes are such as would be expected when the enormous size difference between those of breeding and non-breeding animals is considered.

The relation of the diameter of the spermatatic tubules to the weight of the testes is clearly brought out in fig. 5, in which the mean diameters of the spermatatic tubules are plotted against the cube roots of the weights of testes of 91 animals.

It can be seen that there is a linear relationship between the diameter of the tubules and the dimensions of the testes. A straight regression line was fitted of the form

$$y = 217.4 x - 6.25$$

where y = mean diameter of the spermatatic tubules in μ and

$$x = \sqrt[3]{\text{weight of the testes in gm.}}$$

This regression was found to be highly significant.

The size of the interstitial cells was examined in 19 animals. It was found that the mean area of the cells bore a direct relation to the weight of the testes, as can be seen from fig. 6 in which the mean area of the cells is plotted against the square root of the weight of the testes.

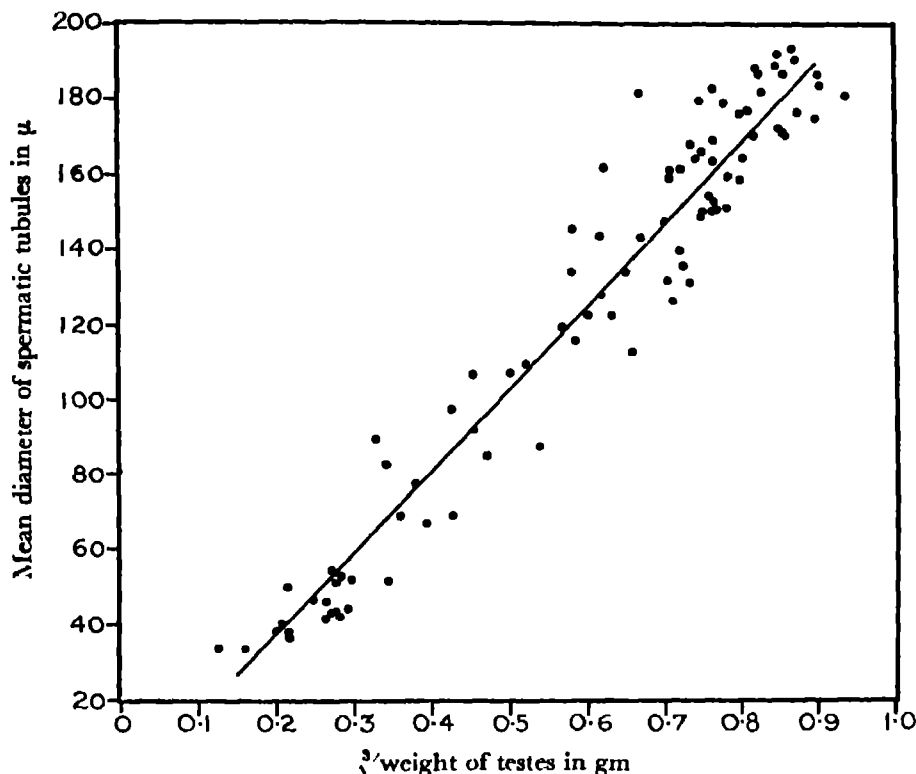


FIG. 5—Scatter-diagram showing the relation of the diameter of spermatic tubules to the weight of the testes. The mean diameter of the spermatic tubules in μ is plotted against the cube root of the weight of the testes. The straight regression line which was fitted to these points is given.

A straight regression line was fitted to these data and was found to be significant. The regression line is of the form

$$y = 100.68 + 12.06 x$$

where y = the mean area of the interstitial cells in sq μ and

$$x = \sqrt[3]{\text{weight of the testes in mg.}}$$

VI—ACCESSORY SEXUAL ORGANS

The size changes in the epididymides, penis, Cowper's (bulbo-urethral) glands, seminal vesicles and prostate combined, and the prepucial glands were examined. It was found that in all these organs the closest correlation exhibited was with the

weight of the testes. The data are given in consequence in the form of correlation tables of the weights of the organ and the weights of the testes.

a. Epididymides

These organs exhibit a close correlation with the weight of the testes. It can be seen from Table IV that this relation approximates to a straight line regression and that the spread is small.

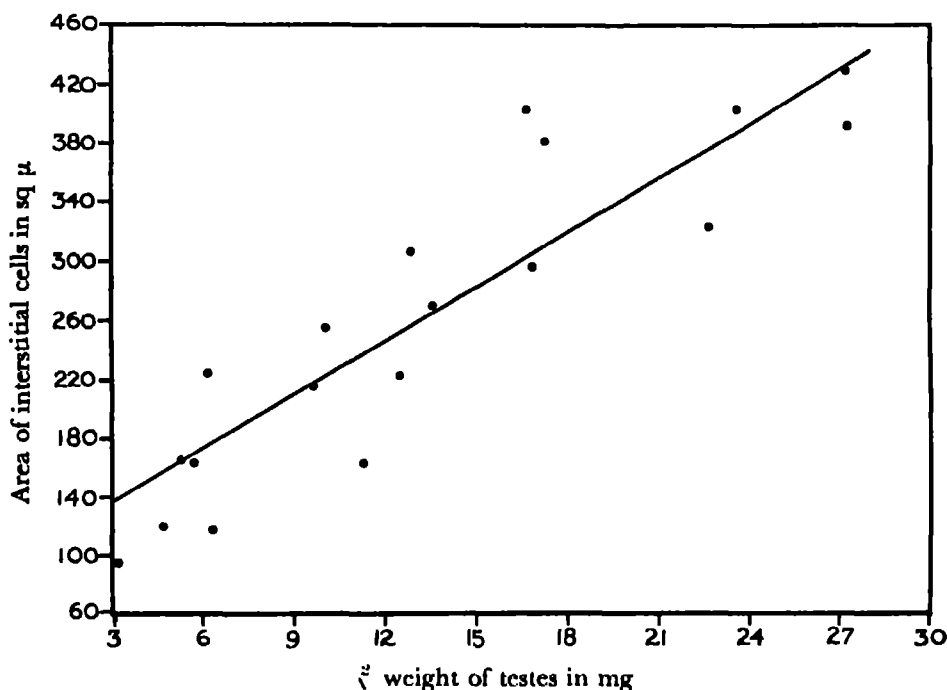


FIG. 6.—Scatter-diagram illustrating the relation between the size of the interstitial cells of the testis and the weight of the testes. The area of the interstitial cells in sq μ is plotted against the square root of the weight of the testis. The straight regression line for these points is given.

Sections of the epididymides of a large number of animals were examined for the presence of spermatozoa, particular attention being paid to those of animals taken in spring, at the onset of the breeding season in which spermatozoa were present in the testes, and of mature animals which were regressing during September, October, and November, and in which spermatozoa were absent from the testes. All animals with testes under 200 mg in weight in which spermatozoa were present during March and the single animal at the end of February were examined and were found with one exception to have spermatozoa in the epididymis. This exception (E 400) was obtained on March 4, and was the only animal with spermatozoa in the tubules in which the testes weighed under 100 mg. All the other animals examined throughout the season in which spermatozoa were present in the testes also had spermatozoa in the

epididymides. It is clear from these results that mature spermatozoa can be found in the epididymides almost immediately after their appearance in the testes. The presence of mature spermatozoa in the testes may, therefore, be taken for practical

TABLE IV

[illegible]

purposes, to indicate their presence in the epididymides. The epididymides of 22 animals obtained in September, October, and November that were definitely adult but in which spermatozoa were not found in the testes were examined. It was found that spermatozoa were present in the epididymides of 13 of these, 12 obtained during September and October and 1 in November, but were absent from the other 5 obtained during the former months and from 4 of those obtained in November. The data are given in Table V. It is apparent, therefore, that the

spermatozoa persist in the epididymides for a considerable time after they have disappeared from the testes, as is known to occur normally after castration in a number of animals.

TABLE V—ADULT ANIMALS IN AUTUMN IN WHICH SPERMATOZOA WERE NOT FOUND IN THE TESTES

Ref. No.	Date	Weight of testes mg	Spermatozoa in epididymides
	September		
140	16	18	0
901	17	24	+
907	18	22	+
908	18	18	+
915	20	26	+
927	21	30	+
168	24	21	+
	October		
936	4	30	+
939	4	22	+
941	4	39	+
954	5	77	+
959	5	8	0
977	7	20	0
978	18	76	+
979	18	30	+
981	18	12	0
989	19	40	0
	November		
1014	10	22	0
1025	12	20	0
1027	13	28	0
1031	15	16	+
205	23	12	0

b. Penis

It is obvious from Table VI that the relation of the weight of the penis to the weight of the testes approximates to a straight regression line. The spread is wider than for the epididymides, as would be expected from the greater difficulty of accurately separating the penis from its attachments.

c. Cowper's Glands

Although the correlation of the weight of these glands with that of the testes is not so close as with the epididymides and penis, it is sufficiently obvious from Table VII that a similar relation exists.

TABLE VI

[illegible]

TABLE VII

24						1				
23							1			
				1				3	2	
16					1			5		1
15				1	5	8	10	4		
		1		1	4	6	20	6	4	1
			1		4	14	25	18	3	
8			2		4	12	22	19	1	1
7			1	9	8	13	6	4		
	1		3	6	6	8	1			
	2	5	7	2						
0	1	1	1	1						
	0			0.49	0.5				0.99	1.0
	Weight of testes in gm									

d. Seminal Vesicles and Prostate

The relation of the weight of these organs to the weight of the testes is not so simple as for the other organs described above. Examination of the data showed that the relation existing during the spring months when the seminal vesicles and prostate were growing rapidly, was different from that exhibited in autumn when the organs were regressing. The data for the months of January to May inclusive, are given in Table VIII. It can be seen that the relation of the weight of the seminal vesicles and prostate to the weight of the testes falls into two clearly defined phases. During the first phase, extending until the testes have attained a weight of approximately 250 mg, the seminal vesicles and prostate do not exhibit any significant increase in weight. In the subsequent phase rapid growth in weight correlated with that of the testes occurs. During both phases the relation evidently approximates to a straight line regression although the spread in the second phase is considerable. It is apparent, therefore, that the growth of the seminal vesicles and prostate relative to that of the testes at the onset of the breeding season exhibits a marked lag, and does not, in fact, begin until the testes have attained a comparatively large size and considerably after the stage at which mature spermatozoa are found both in the testes and epididymides. Since no animals with testes 250 mg in weight and over were obtained before March 24, and all animals obtained after April 16 and before the appearance of young animals of the season had testes over this weight, it shows that the growth of the seminal vesicles and prostate begins during this period.

The data for the months of September and October, when the reproductive organs of mature animals are rapidly regressing at the close of the breeding season, are given in Table IX. It can be seen that the relation of the weights of the seminal vesicles and prostate to the weights of the testes during this period is not divisible into two phases but approximates to a single straight regression line.

A similar type of correlation between the growth and regression of the testis and that of the accessory organs is found in the hedgehog (ALLANSON, 1934).

e. Prepuccial Glands

The relation of the weight of the prepuccial glands to the weight of the testes appears to be similar to that of the weight of the seminal vesicles and prostate to the testes and the data are consequently treated in a similar manner. The data for January to May inclusive are given in Table X and exhibit a similar, though less clearly marked, lag in the growth of the gland in relation to the testes. The data for September and October, given in Table XI, are unfortunately scanty but serve to show that the prepuccial glands do not regress more rapidly than the testes, as would occur if the relationship was similar to that for the spring months. It is probable, therefore, that the weight of the prepuccial glands bears to the weight of the testes during September and October a simple relationship such as that exhibited by the seminal vesicles and prostate.

TABLE IX—SEMINAL VESICLES AND PROSTATE (SEPTEMBER AND OCTOBER)

[illegible]

TABLE X—JANUARY TO MAY (INCLUSIVE)

Weight of prepuccial glands in mg	99						1	6		
						1	1			
						1	4	2	1	1
						2	5	6	3	
	50			1		4	12	10	2	1
	49			1		5	7	8	3	
						4	5	5		
				1	1	5	10	2	1	
			2	3	3	4	7	1	1	
0	3	5	4	2	3	6	4			
	0				0.49	0.50			0.99	1.0
Weight of testes in gm										

TABLE XI—SEPTEMBER AND OCTOBER

Weight of prepuccial glands in mg	99									
	50									
	49			1						
					1	2				
		1				2	1			
		1	1	3	3	1	1			
0	21	1								
	0					0.49	0.50		0.99	1.0
Weight of testes in gm										

I am indebted to Professor F. W. ROGERS BRAMBELL for the use of the material and to him and Dr. A. S. PARKES, F.R.S., for their advice and criticism. The expenses of this research were defrayed in part by grants from the Government Grant Committee of the Royal Society to Professor BRAMBELL, for which I wish to express my thanks.

VII—SUMMARY

The material consists of 593 male *Evotomys* of which 583 have been employed in describing the seasonal variation in the body-weight and in the reproductive organs.

Sexual activity is more closely correlated with the weight of the testis than with the body-weight, since mature spermatozoa are found in almost all males with testes

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weighing 100 mg or more. This is a more accurate criterion of fecundity than the body-weight.

Sexual activity commences early in March and is accompanied by an increase in body-weight and enormous growth of the testes from a winter weight of less than 40 mg to a mean summer weight of 682 mg.

Young males born early in the breeding season become sexually mature before the end of the same season, whereas those born late in the season do not attain sexual maturity until the following spring.

In late August, September, and October the testes are in regression and by November there is complete cessation of spermatogenetic activity.

All male *Evotomys* during the winters 1931-32 and 1932-33 were aspermatic during November, December, and January, and the first three weeks of February. During this period two types of testes could be distinguished histologically; those of young animals that had not bred and those of adults which had atrophied.

The size of the spermatogenic tubules and interstitial cells were found to be directly and simply related to the size of the testes.

Seasonal changes occur at the same time in the accessory organs. When correlated with testis weight they appear to fall into two groups. The epididymides, penis, and probably the Cowper's glands exhibit a straight line regression throughout the year, while the seminal vesicles, together with the prostate and the prepuce glands in the spring, do not increase in weight until the testes have attained a weight of 250-300 mg. Subsequent growth in these organs is very rapid and is correlated with testis growth.

The male accessory organs resemble those of the common mouse (DISSELHORST, 1904) except in details. A glandular urethral sinus is present in the bulbus which resembles that of the mouse in structure and position.

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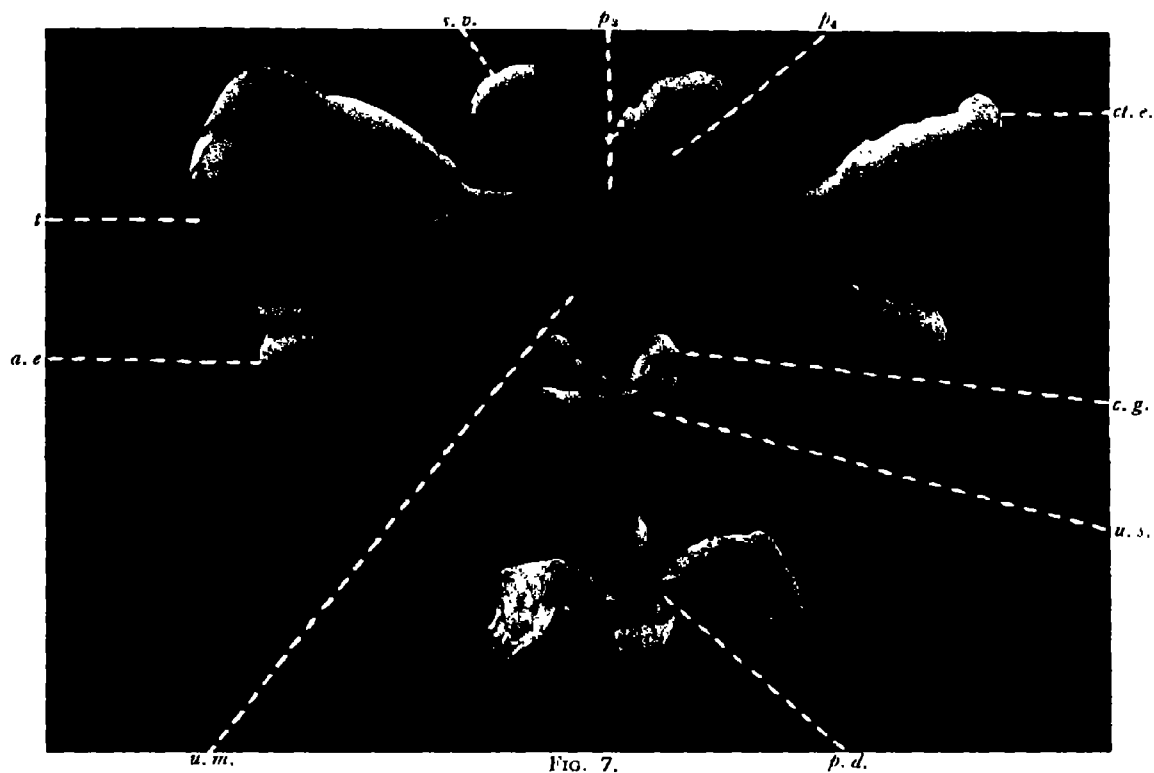


FIG. 7.

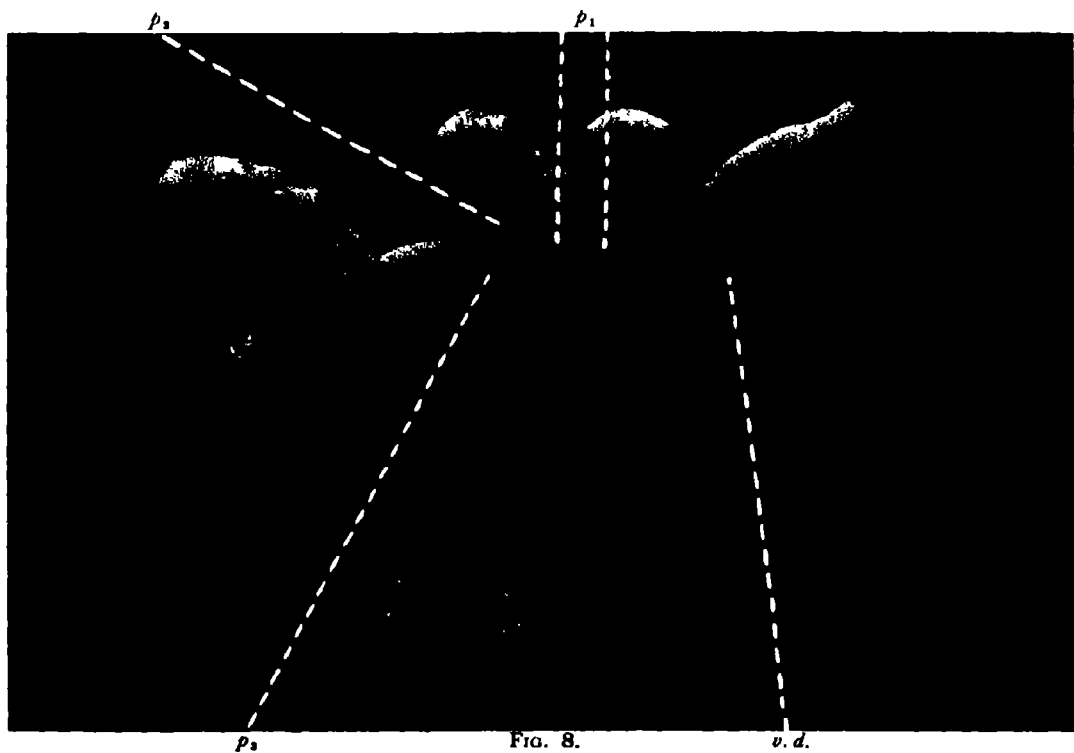


FIG. 8.

PLATE 13.

- FIG. 9—Dissection of the reproductive organs of the male *Eutamias* during the breeding season. Ventral view. $\times 3.0$. The bladder has been pulled backwards so as to show the ampullary glands at the base of the vasa deferentia. The large mass of bulbo-urethral muscle is seen partly obscuring the Cowper's gland on the right side.
- FIG. 10—Transverse section of the testis of immature *Eutamias* (E 306) in winter (3 Jan. '32). The large number of closely packed spermatogenic tubules, surrounded by a thin tunica albuginea, is characteristic of this stage. $\times 68$.

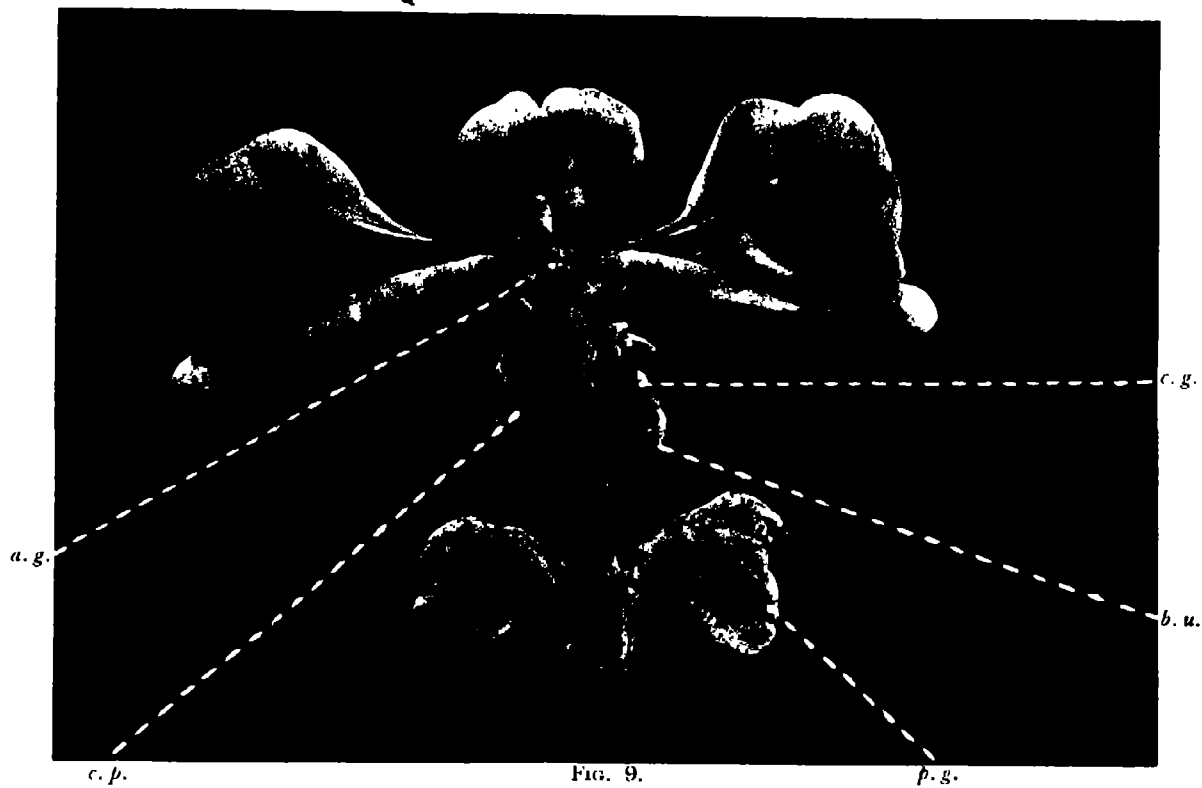


FIG. 9.

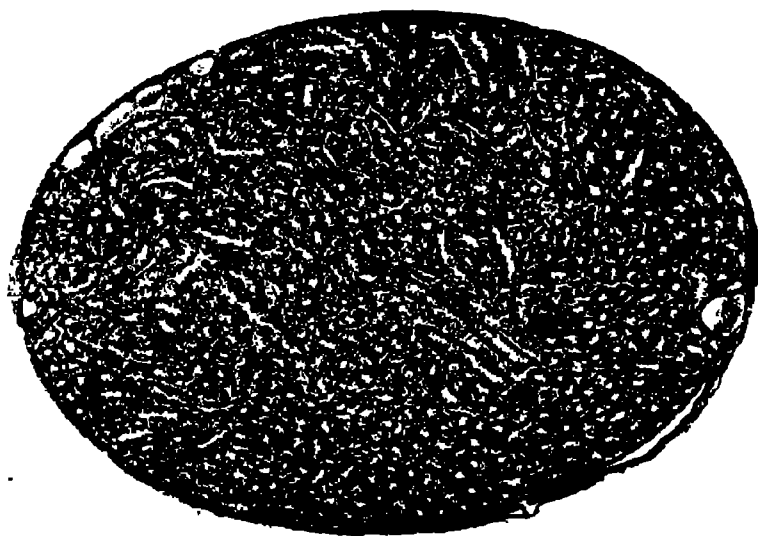


FIG. 10.

PLATE 14.

- FIG. 11—Part of section of testis shown in fig. 10. The tubules contain few spermatogonial nuclei and a large number of Sertoli nuclei. $\times 260$.
- FIG. 12—Part of a transverse section of the testis of an adult *Eutamias* (E 469) at the onset of the breeding season (26 Feb. '32). Primary spermatocytes in pachynema are again to be seen. This shows that the male *Eutamias* can breed in one season, spend the winter in a state of regression and again become active in the following breeding season. $\times 150$.
- FIG. 13—Part of section of testis shown in fig. 15. The characteristic zone of eosinophil cytoplasm between the wall of the tubule and the ring of Sertoli nuclei is seen. $\times 300$.
- FIG. 14—Part of a transverse section of the testis of an immature *Eutamias* (E 412) at the onset of the breeding season (11 Mar. '32). Primary spermatocytes in pachynema are seen in all the tubules. $\times 150$.



FIG. 14.

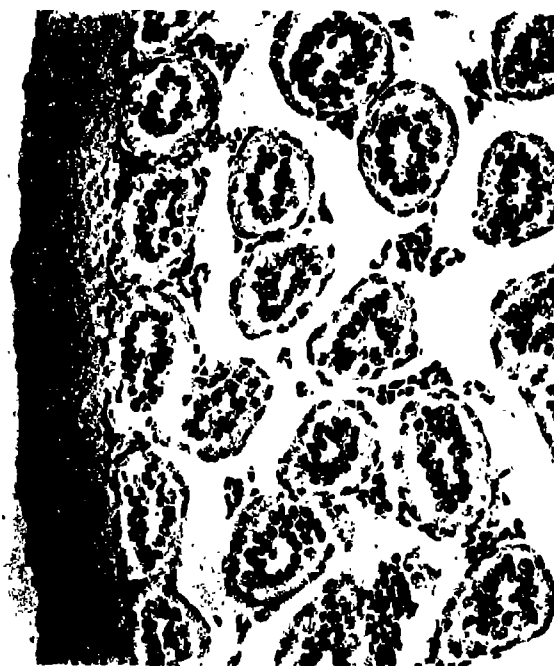


FIG. 13.

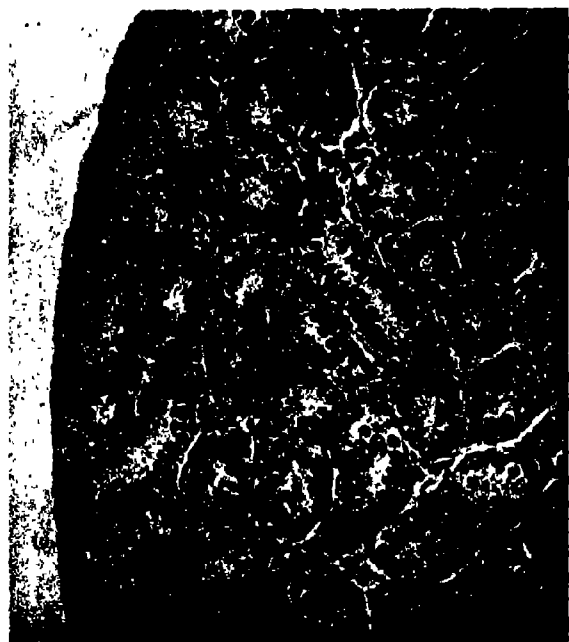


FIG. 11.

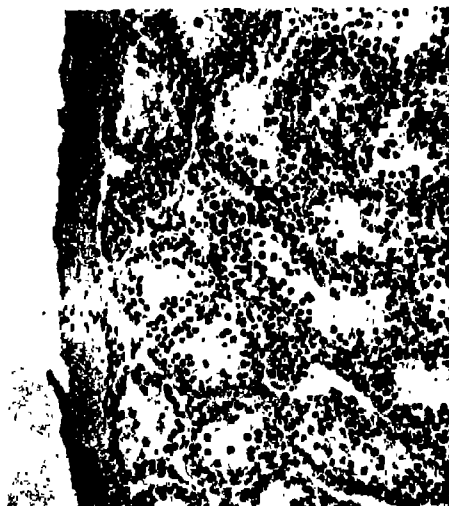


FIG. 12.

IV—On the Tooth-replacement in Theriodont Reptiles

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(Communicated by J. STANLEY GARDINER, F.R.S. —Received July 31—Read November 14, 1935)

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I —INTRODUCTION

In describing the very mammal-like dentitions of some of the advanced Theriodont reptiles various authors have identified premolars and molars (BROOM, 1913). Though there is some evidence for this differentiation in the form of the teeth of some of the gomphodont cynodonts, there is no satisfactory evidence that only two dentitions were present or that the replacement was of a mammalian type.

On the other hand SUSHKIN (1927) described the type of the primitive cynodont *Permocynodon sushkini*, SMITH WOODWARD, as having several molariform teeth "changing" and says "it may be established thus that the teeth are changing irregularly as usually in the reptiles and that there is no difference in this respect between the anterior and hindermost teeth ; thus despite the difference of the form, no premolars and molars can be distinguished". Unfortunately he does not say which teeth are changing.

Other authors, uncertain of the manner of replacement, have avoided implying a mammalian succession by the use of such terms as "cheek" teeth or "post-canine" teeth.

The difficulties of analysing the dentitions of such fossil groups are due to the rarity of specimens in which replacement of the teeth is actually taking place, and

also to the lack of developmental series. In this respect it is fortunate that there is a primitive cynodont, *Thrinaxodon liorhinus*, SEELEY, which is relatively abundant. One specimen of this animal, which is in the Museum of Zoology at Cambridge, seemed to show the method of tooth-replacement. An examination of all the available material of the species was made and this yielded sound evidence that the replacement is not of the mammalian type, but that alternate teeth are replaced at one stage and the others later. This condition is apparently present in other members of the group, and is an inheritance from the earliest tetrapods, a conclusion which is in accordance with BOLK's interpretation of the condition in living reptiles.

II—NOTE ON THE GENERA *Nyctosaurus* AND *Thrinaxodon*

It has long been recognized that the cynodonts *Nyctosaurus larvatus*, OWEN, and *Thrinaxodon liorhinus*, SEELEY, are very closely related. When describing the type of the latter SEELEY (1894) stated that it had six post-canine teeth each with three cusps, whereas OWEN's *Nyctosaurus larvatus* had seven or eight post-canine teeth and several of them had four or possibly five cusps.

In a paper on *Thrinaxodon liorhinus* (PARRINGTON, 1933), the writer described one specimen in which a seventh post-canine tooth was present in one maxilla, and a specimen in which there were seven and eight post-canine teeth on the two sides respectively.* Subsequently two further skulls have been seen in which seven teeth are present, one in the British Museum (Natural History), R.5480, and one in the Transvaal Museum—the anterior of two skulls in a block numbered 80.

In regard to the question of tooth cusps, HAUGHTON (1924) reported a specimen of *Thrinaxodon liorhinus* in which one tooth had an additional cusp on the inside. BROOM (1932), however, states that he has never seen a specimen in which the teeth had more than three cusps. A specimen collected by the author in 1933 showed very plainly that the fourth tooth on either side had a fourth cusp, lying posterior and slightly lingual to the third. Careful preparation has shown the presence of additional cusps on the teeth of the maxillae of three other specimens, fig. 1. These cusps have been seen on the posterior inner edge of the fourth tooth in one specimen, C; on the anterior inner edge of the fifth tooth of two specimens, D and F; and on the anterior and posterior sides of the fourth tooth and the anterior of the fifth tooth of another, E. The apparent absence of these cusps in other specimens is probably due to the difficulty of cleaning between the teeth when they are set close together, particularly when the matrix is not relatively soft. The teeth of the left maxilla of specimen D were removed, the fourth being destroyed in the process. It could then be seen that the fifth tooth had only the additional anterior cusp, and that none was present on the first, second, third, or sixth tooth. The

* This second specimen has much smaller teeth than those of any other I have examined and, since it is too badly preserved to give any information on the question of tooth-replacement, it is omitted from this discussion.

dissection also exposed the teeth of the left dentary, fig. 1, and showed that the fifth and seventh teeth have five cusps as has, probably, also the eighth. The fact that the type of *Nythosaurus larvatus* consists largely of a negative mould makes it difficult to be certain of its detailed characters, and this further information on the teeth of *Thrinaxodon liorhinus* seemed to remove the justification for separating the two genera.

There are, however, sufficient fixed points shown in the type of *Nythosaurus larvatus* to enable a reconstruction to be made. These points are :—the position of

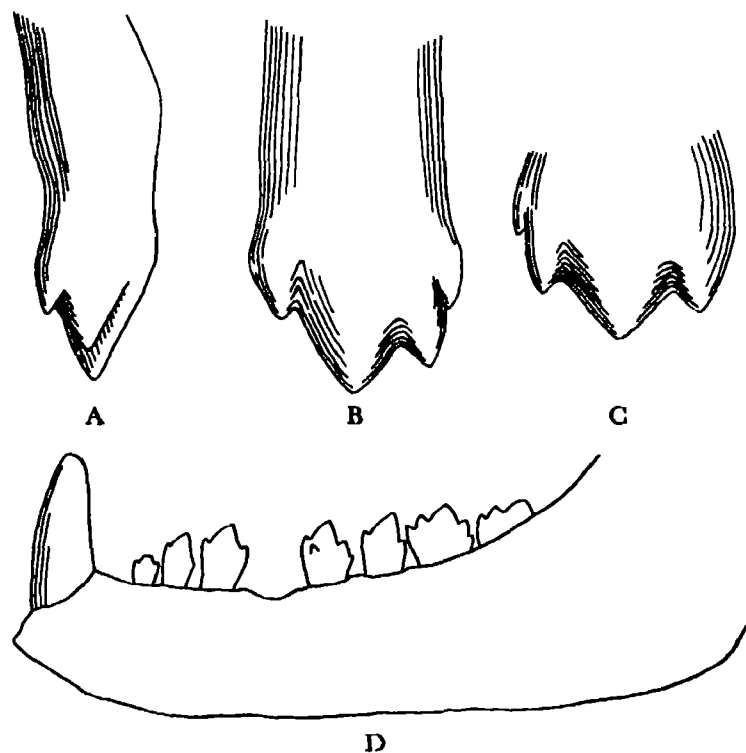


FIG. 1—Teeth of *Thrinaxodon liorhinus*, SEELEY. A, B, anterior and inner views of the fifth left post-canine of specimen D. X.12; C, outer view of right fourth post-canine of specimen C. X.12; D, left dentary of specimen D. X.3.

the canine tooth and the quadrate in the left side; the anterior border of the orbits; and the position of the pineal foramen; the back of the parietal crest; and of the foramen magnum as shown by the cast of the brain case. The position of the back of the parietal crest is given by the mould of the recess in the inter-parietal in the type compared with the section of a skull of *Thrinaxodon liorhinus* (PARRINGTON 1935).

When a reconstruction of *Nythosaurus larvatus* based on these points and on the mould of the nose is compared with one of *Thrinaxodon liorhinus* certain differences are at once apparent, fig. 2. The most obvious of these is the position of the quadrates,

which in *Thrinaxodon* are nearly in line with the pineal foramen while in *Nyctosaurus* they are only just in front of the back of the parietal crest. In addition *Nyctosaurus* has the occiput sloping much more, and is relatively shorter anterior to the pineal foramen. The reconstruction of a series of specimens of *Thrinaxodon liorhinus*, fig. 3, shows that the ratios of the distances between the back of the parietal crest and the pineal foramen, of the pineal to the front border of the orbits, and from the front border of the orbits to the tip of the snout vary between 1 : 1.83 : 2.25 in a small specimen to 1 : 1.76 : 2.11 in a large specimen, while the ratios in *Nyctosaurus* are 1 : 1.6 : 1.86—the last figure being made on the estimated length of the snout. Another point of difference lies in the position of the post-canine teeth, the last

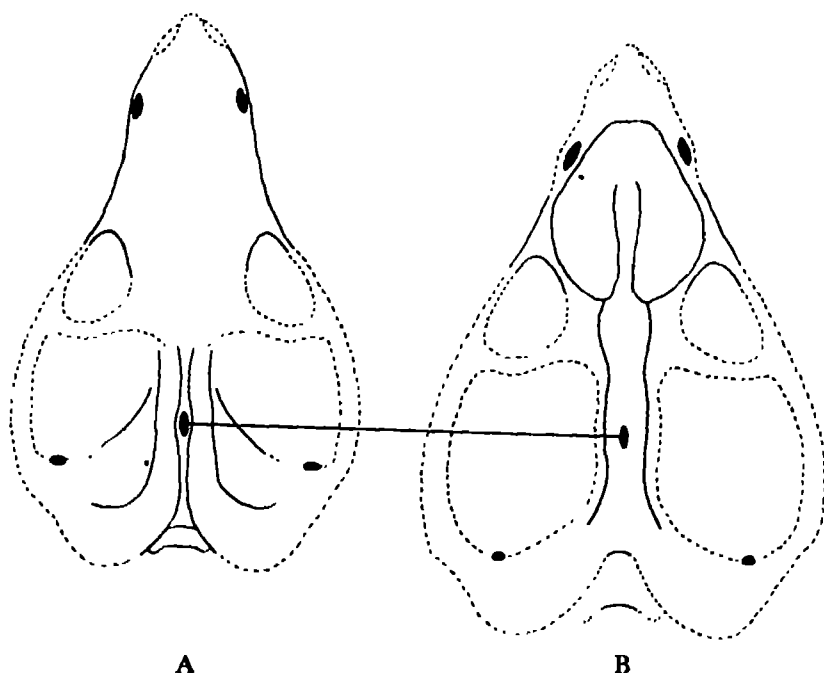


FIG. 2.—Restorations of A, *Thrinaxodon liorhinus*, SEELEY, and B, *Nyctosaurus larvatus*, OWEN. Natural size. The positions of the canines, pineal foramina, and lower ends of the quadrates are marked in black.

three of which are situated below the orbit in *Nyctosaurus*, whereas only the sixth and, when present, the seventh occupy this position in a *Thrinaxodon* of similar size. It seems quite clear therefore that the two genera are distinct.

III—ON THE TOOTH-REPLACEMENT IN *Thrinaxodon liorhinus*, SEELEY (a) *Material*.

Nine specimens of *Thrinaxodon liorhinus* are described in this paper, three the property of the British Museum (Natural History) (B.M.N.H.), and six belonging to the University Museum of Zoology at Cambridge (C.M.Z.). For convenience of

discussion they have been arranged in order of their size and given a letter. This material is as follows :—

Specimen A, C.M.Z. R.2733 ;	Specimen F, C.M.Z. R.2734 ;
B, „ R.2739 ;	G, B.M.N.H. R.5480 ;
C, „ R.2737 ;	H, C.M.Z. R.2736 ;
D, „ R.2738 ;	I, B.M.N.H. R.511a.
E, B.M.N.H. R.3731 ;	

All the Cambridge material was collected from the *Lystrosaurus* zone (*L. Trias*) at Harrismith in the Orange Free State, as was the British Museum specimen No. R.5480. The remaining two specimens are registered as Lower Trias, number R.511a from the Orange Free State and number R.3731 from Griqualand.

In order to establish the relative stages of growth of these specimens it was necessary to make reconstructions as far as this was possible. Though it is seldom that an absolutely complete or uncrushed specimen is found—the premaxillae and jugal arches are often missing—the presence of such parts as the septomaxillary foramen, the anterior borders of the orbits, the quadrates, and the occiputs enable restorations of six specimens to be made with fair accuracy. Unfortunately the three specimens A, D, and I, are too damaged in the region of the brain case to enable useful restorations to be made, but their growth stages are given by their dentaries. The growth series of the six specimens show a number of points of interest, fig. 3. If the drawings of the smallest and the largest specimens are placed with their pineal foramina on a line, and such points as the back of the parietal crest, the anterior border of the orbits, and the tip of the nose are joined, it will be found that the drawings of the remaining four skulls conform to these lines with only two exceptions. In specimen G the anterior border of the orbit falls somewhat short, but the specimen is distorted and the explanation of this may well lie in faulty reconstruction. A more interesting variation is found in specimen E, where the skull conforms perfectly only if the pineal is placed just behind the line of the other pineals. Also the nose of this specimen is a little broader than that of the slightly larger specimen F. Such differences may be due to natural variation or possibly sexual dimorphism.

This series shows the changes in proportion of the skull which take place during growth. The increase in the length of the skull is made up of about 42% in the increase in the nose anterior to the orbits, 35% in the region between the pineal foramen and the front of the orbits, and 23% between the pineal and the back of the parietal crest. Also while the length has increased by about 35% the width has increased by about 50%.

Restorations in side view are much less satisfactory owing to the fact that the majority of skulls have been crushed. Specimens H and F are very little distorted, however, and a fairly reliable restoration can be made of specimen C, fig. 4.

It will be seen from this series that the canine tooth occupies a constant position, and the post-canines remain close to it and do not occupy a much greater space in the old specimens than they do in the young. The result of this is that whereas in the

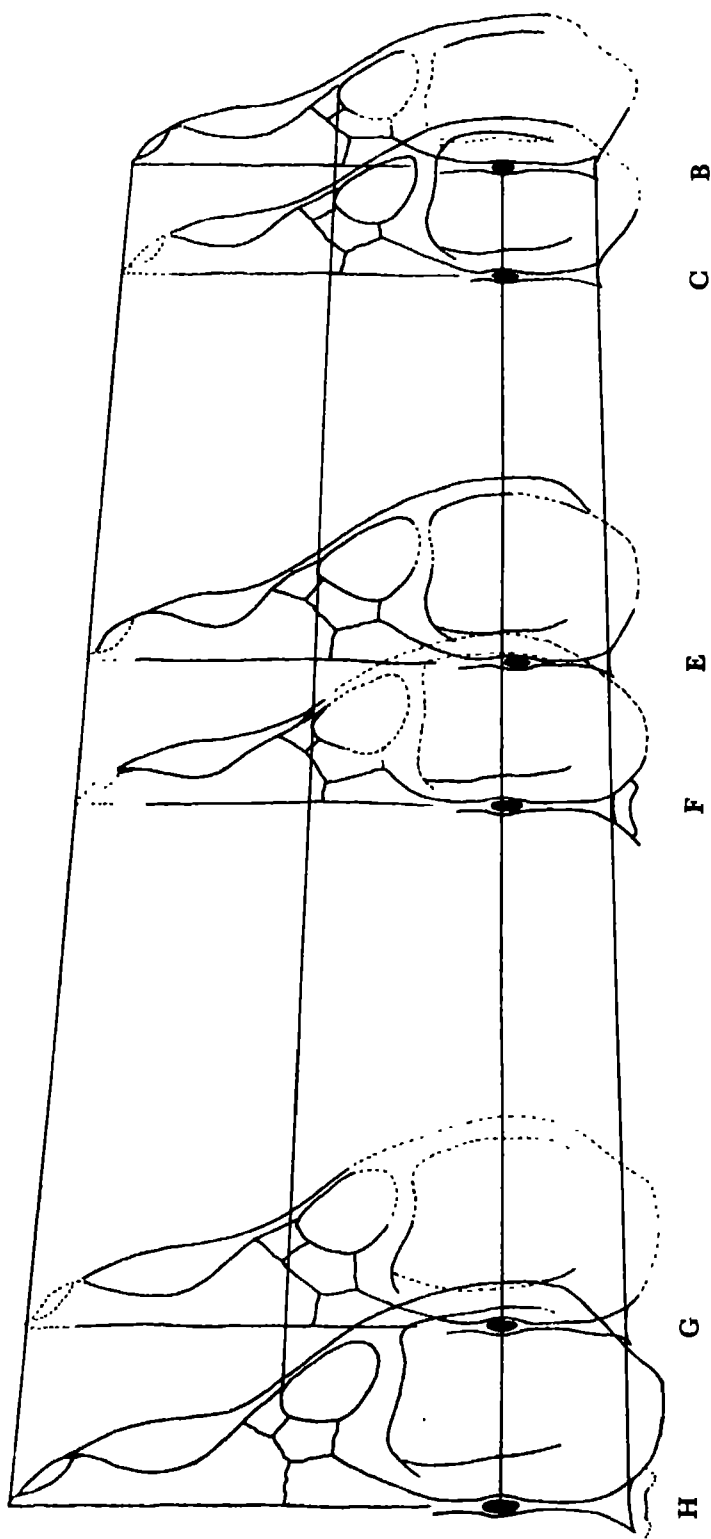


FIG. 3.—Restorations of the skulls of *Thrinaxodon liorhinus*, SEELEY, to show the change in proportions during growth. Natural size.

young forms the posterior three teeth are situated below the orbit, only the last tooth occupies that position in the oldest. Another point of interest is in the position of the quadrates which occupy a much more posterior position in the young than they do in the older forms. This position of the quadrate, together with that of the last post-canines, and the fact that the young have a relatively small nose, suggests that it would be hard to distinguish them from the young of *Nyctosaurus*.

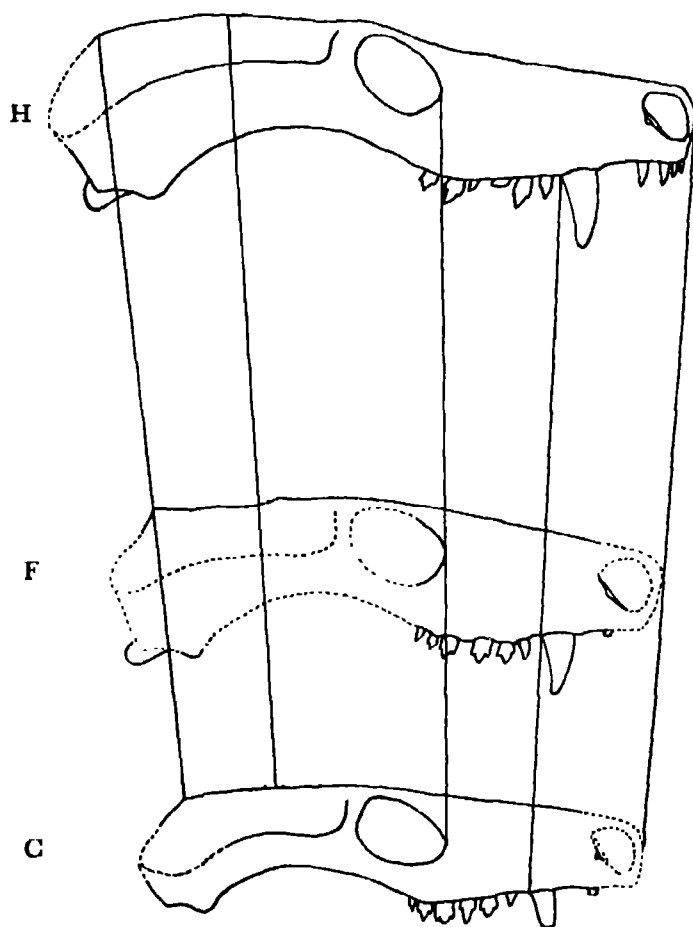


FIG. 4—Restorations of the skulls of *Thrinaxodon liorhinus*, SEELEY, in side view. Natural size.

(b) *Tooth-replacement*

The first indication that the manner of the replacement of the post-canine teeth could be seen in these forms was noticed in specimen F. This is the second specimen that was described in 1933 when the presence of seven teeth in the left side was noted and the suggestion made that the extra tooth was actually the fourth. Comparison of the two sides shows that the anterior three teeth are almost identical with their fellows in the opposite side, as are the last three. The distance occupied

is the same in both maxillae but in the right side there is a very marked gap in the middle of the series. The interpretation now suggested is that the real fourth tooth is missing from the right side, and that the true seventh is present in both maxillae. This is strongly supported by the fact that in every specimen examined—including a large number in various museums in South Africa—the fifth tooth is always relatively large, as in the left side, and is not of the small size of the actual fifth in the right maxilla. A similar condition may have been present in specimen A. Here the right maxilla is separate and has been cleaned in palatal view. The tip of the replacing canine, visible in its socket, and the root of the second post-canine tooth are all that is preserved, but it is seen that the alveoli of the three anterior post-canine teeth are confluent as are the last three, while the two series are separated by a distinct bridge of bone, fig. 5A. The left maxilla has the second, third and fifth teeth in place but there is no indication of a similar gap between the third tooth and the alveolus of the fourth. It is not possible to say whether or not a seventh tooth was present in this side. The explanation of the absence of the true fourth tooth in specimen F is not at all clear, but it is important to recognize that the teeth which are present in the right maxilla are the first, second, and third, and the fifth, sixth, and seventh. A further point in support of this interpretation, and the clue to the manner of the tooth replacement, is given by the alveoli. The nature of these is shown very clearly in this specimen, and there can be no doubt that their characters are true and not due to the preparation. The teeth and bone were very clear and distinct from the matrix which was sufficiently soft to enable a very satisfactory preparation to be made. In both sides the alveoli of the first, third, fifth, and seventh teeth are large and the teeth are only loosely implanted in them; while the second, fourth, and sixth teeth in the left side and the second and sixth in the right side are held tightly, the bone coming closely round their roots, fig. 5B and C. The space of the missing fourth tooth in the right side is occupied by bone which reaches downwards between the large alveoli of the third and fifth teeth in the manner of the bone round the roots of the second and sixth teeth. The obvious interpretation of this difference in the nature of the alveoli of the odd and even numbered teeth is that those in the loose sockets are replacing-teeth that had erupted only a short time before the animal died.* The fact that the seventh teeth are implanted in a loose socket apparently means that they have been cut only a short time, but since there is no evidence of seven teeth being present in a smaller animal they are unlikely actually to have replaced teeth.

The examination of the remaining available material provided very satisfactory evidence in support of the foregoing interpretation. The main point of interest in the two smallest specimens lies in there being evidence of replacement of the canines and incisors. In specimen A the tip of a replacing canine is visible in the empty

* The appearance in the left side suggests very strongly that the seventh tooth was not completely erupted. Furthermore, the teeth implanted in the loose sockets are rather larger than the others and have the centre cusp slightly larger in proportion.

socket of the right maxilla, and a small replacing-tooth is seen pressed against the root of the first incisor in the left dentary. The section cut through specimen B exposed a replacing-tooth which appears to be in the process of eruption just behind the first incisor of the right premaxilla. In neither specimen are the post-canine teeth well preserved but a point of interest in specimen B is the fact that the sixth tooth does not appear to have erupted in the right maxilla.

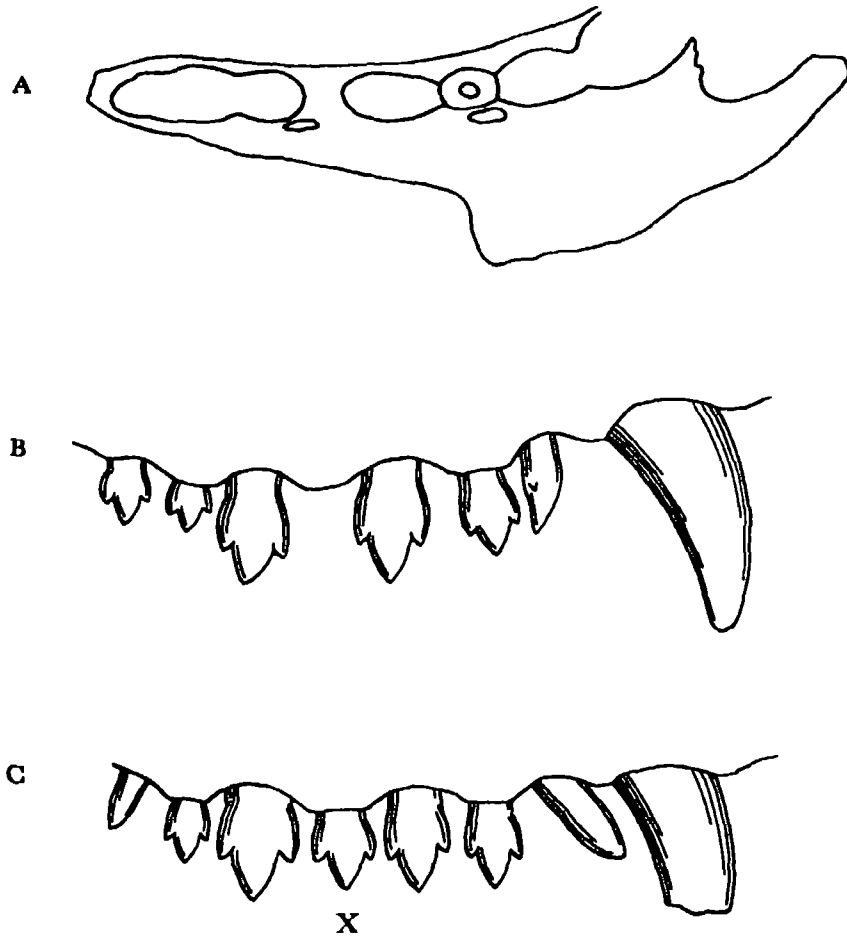


FIG. 5—*Thrinaxodon liorhinus*, SEELEY. A, right maxilla of specimen A, palatal view; B, teeth of right cheek of specimen F; C, teeth of left cheek of specimen F, drawing reversed for comparison; X, true fourth tooth missing in right maxilla. All $\times 4$.

Specimen C has very large alveoli for the first post-canines and, while the tooth is missing in the right side, there is apparently a replacing-tooth half erupted in the left. Also the third teeth in both sides have the appearance of being incompletely erupted, and have their centre cusps rather larger in proportion to the main cusps in the adjacent teeth. The suggested interpretation of this dentition is that the

first post-canine teeth are being replaced while the third may have already been replaced, fig. 6.

The next specimen, D, is very poor. Its position in the series is given by the size

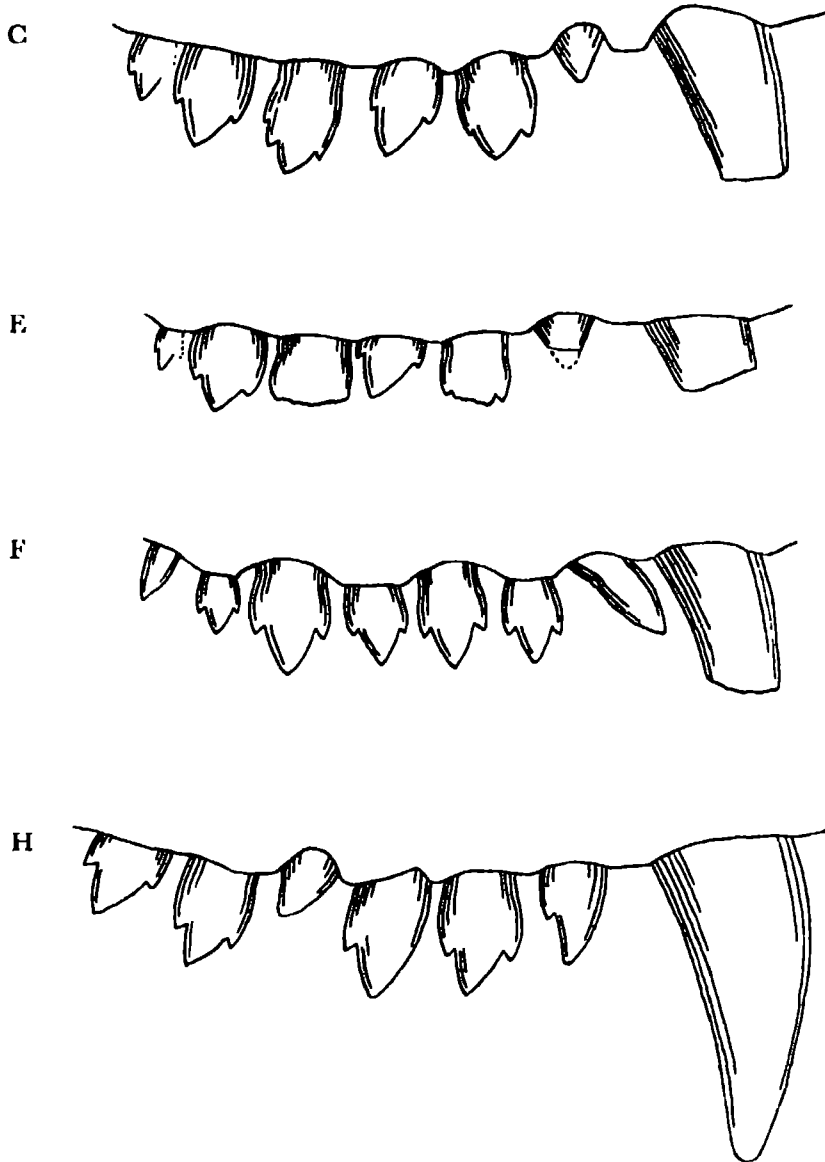


FIG. 6.—*Thrinaxodon liorhinus*, SEELEY. Cheek teeth of specimens C, E, F, and H, x 4. Teeth missing or damaged in the right maxilla have been drawn from the left, and F has been reversed from the left side.

of the dentary which is slightly larger than that of specimen C. In neither case, however, is this bone well preserved. Here the post-canine teeth of the right side appear to show, though not so clearly, the same behaviour as that described for

specimen F. The first and fifth teeth were certainly very loose and half out of their sockets. The teeth of the left maxilla were dissected out in the hope of finding replacing-teeth but no trace of these was found. The interpretation here is that the first, third, and fifth post-canine teeth have been replaced, and that the replacing-teeth of the second, fourth, and sixth teeth were not sufficiently calcified to be preserved.

Specimen E,* the next in order of size, is very interesting. It can be seen quite clearly in both sides that the first and third teeth are half erupted, the fifth teeth giving no convincing evidence of having been replaced. The crowns of the second and fourth teeth are not well preserved, but their appearance suggests that the cusps were subequal while the replacing third teeth have relatively large centre cusps, fig. 6. The sixth tooth has not erupted in the left side.

Specimen F has already been described, and the interpretation suggested that the first, third, fifth, and seventh teeth have just erupted.

Specimen G is an old one and the teeth have been prepared very badly. The following points are of interest, however. In the right side the fourth post-canine tooth seems to be more tightly clasped by the bone than the others, the sixth tooth (as shown in both sides) is small, and a seventh, also small, is present in the left maxilla.

Specimen H is of considerable importance. In both maxillae the fourth tooth is plainly just erupting—only the centre cusp being visible, fig. 6. Additional points are the fact that while the first three teeth are very similar to those of specimen F the sixth tooth is very much larger than in any of the smaller specimens. There is no evidence for a seventh tooth having been present. An important point in the dentition of this specimen is that the second incisor of the left premaxilla is smaller than that of the right, and may be erupting.

In the largest specimen, I, the post-canine teeth are rather damaged. The fourth and sixth teeth are seen to be as large as in specimen H and, what is of most importance, the second tooth of the right side has a relatively enormous cusp and has the appearance of being incompletely erupted.

This evidence of replacement may be summed up as follows:—The canine is being replaced in the first animal, which is certainly very young, and the first upper incisor in the next, an animal with a skull length of 61 mm.†

Of four skulls varying between 61 and 71 mm, C shows the first post-canine tooth replacing, and E the first and third post-canines, while D and F suggest that the first, third, and fifth have already been replaced.

In the three skulls between 79 and about 85 mm the smallest, G, suggests that the second, fourth, and sixth teeth, at least, are the same as those of the smaller specimens; the second, H, shows that the sixth teeth have been replaced (it is the

* This is the specimen mentioned by WATSON (1920) as showing tooth change.

† The skull lengths are given from the premaxillae to the back of the parietal crest as this can be given with the greatest accuracy in most specimens.

first specimen to have this tooth large), and the fourth are just erupting. In the largest, I, the second and sixth teeth have definitely been replaced.

There is sufficient positive evidence in these specimens to show that the canines, and at least one incisor, are replaced in the very young animal ; the first, third, and fifth post-canine teeth when the skull reaches a length of about 65 mm ; and the second, fourth, and sixth post-canines when it is about 82 mm.

The only evidence which suggests that more than one replacement occurs is the incompletely erupted second upper incisor in the adult specimen H. There is no evidence, however, to show that the second incisors were replaced at the same time as the first incisors, *i.e.*, in the very young animal, and it is possible, as will be pointed out later, that there was a considerable time interval between the replacement of the alternate incisor teeth as well as in the post-canines.

IV—ON *Parathrinaxodon proops* GEN. ET SPP. NOV.

While investigating the Karroo rocks of the Ruhuhu valley in the south-west of Tanganyika in 1930, Mr. G. M. STOCKLEY (1932), of the Tanganyika Geological Survey, discovered a number of bone localities at two distinct horizons. The remains collected were described by HAUGHTON (1932) who concluded that the fauna of the lower horizon indicated an Upper Permian age, and that the beds were probably homotaxial with the middle part of the Lower Beaufort Beds of South Africa.

Among a series of Permian Theriodonts collected by the author from this horizon in 1933 was a new cynodont of particular interest for which the name *Parathrinaxodon proops* is suggested. The specimen, which was collected from Stockley's site B. 19, near Kingori mountain, consists of a skull which has lost the jugal arches, the top of the brain case, and the lower jaws. The right side of the face is somewhat distorted, and the state of preservation is also unsatisfactory in regard to the sutures, only a few of which can be identified.

The form of the face is unusual for the great depth of the snout, and the splaying out of the borders of the orbits which cause them to face more forwards than outwards, fig. 7.

The front of the premaxillae, with the internarial processes are missing. Weathering has worn the right side down to expose the sockets of four incisors with a replacing-tooth just erupting in the second. Restoration of the snout suggests that there must have been room for six incisors. As preserved it appears that the lower border of the bone curved upwards towards the front. The left premaxilla is so damaged that it yields no information.

The septomaxillae are partly preserved in both sides and show the presence of fairly large septomaxillary foramina below the back of the external nares.

The maxillae are very deep, somewhat swollen above the canines and extending posteriorly nearly to the orbits. They are pierced by six foramina, four above the base of the canines and two, rather larger, posteriorly.

The nasals slope upwards to meet at an angle in the midline and form a ridge which becomes very pronounced between the orbits where they meet the frontals posteriorly in a deeply crenulated suture. There are two foramina visible in the left nasal just posterior to the highest point of the overlap of the maxilla, and indistinct evidence of several smaller ones anteriorly.

The inner borders of the prefrontals are only distinguishable posteriorly where they lie halfway between the orbits and the midline. In this region the frontals slope downwards to meet the nasals and the frontals and form with them two distinct valleys. The anterior extension of the prefrontals cannot be made out with any certainty, nor the borders of the lachrymals except where the left lachrymal meets the maxilla and the extension of the jugal below the orbit.

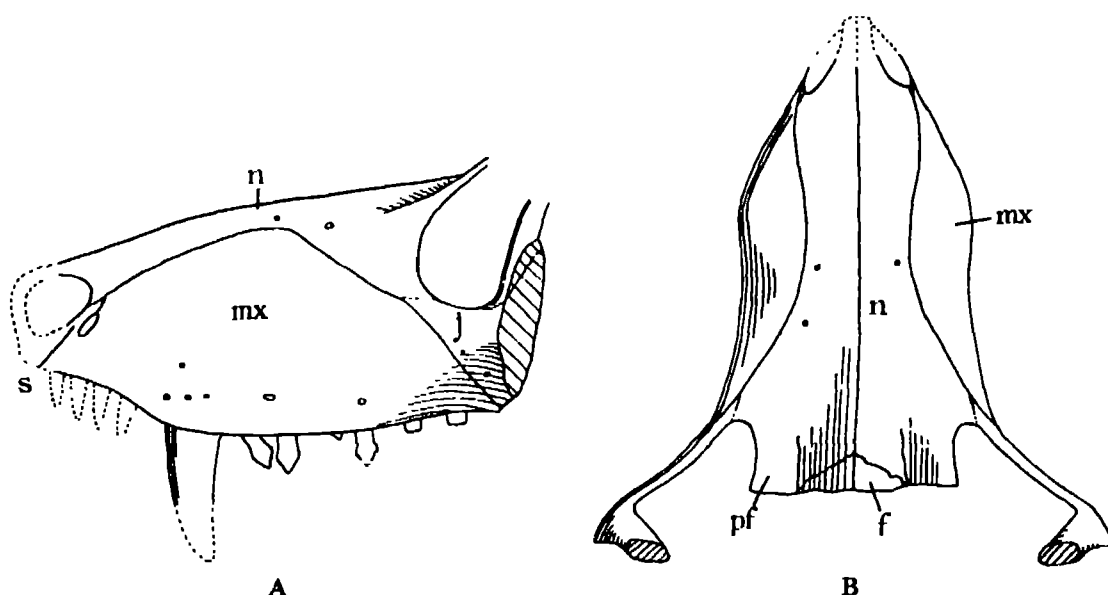


FIG. 7—*Parathrinaxodon proops* gen. et spp. nov. A, side view and B, dorsal view, x 1; f, frontal; j, jugal; mx, maxilla, n, nasal, pf, prefrontal, s, septomaxilla.

The anterior part of the left jugal is preserved. It forms the lower border of the orbit and apparently extends upwards round the back of the post-orbital nearly to the midline. Below the orbit there are two distinct, but small, foramina which face forwards and downwards.

Palate—The bones of the palate are badly fractured and it proved difficult to remove the matrix which will not part readily. Few sutures can be determined but the form is fairly clear.

There is a well-formed false palate, the left side of which has been cleaned, extending backwards nearly to the line of the orbits. Anteriorly there is some indication of palatal processes of the premaxillae, and there are the usual pits for the reception of the lower canines. The maxillae appear not to have joined completely in the midline opposite the canines and for a short distance posteriorly.

The posterior borders formed, presumably, by the palatines, meet at an angle of about 70° and extend backwards to join the ridges of the palatines and pterygoids which formed the walls of the naso-pharyngeal passage.

Part of the vertical plate of the fused vomers can be seen extending backwards to the middle of the skull, but its anterior extension and the outline of the bone is uncertain.

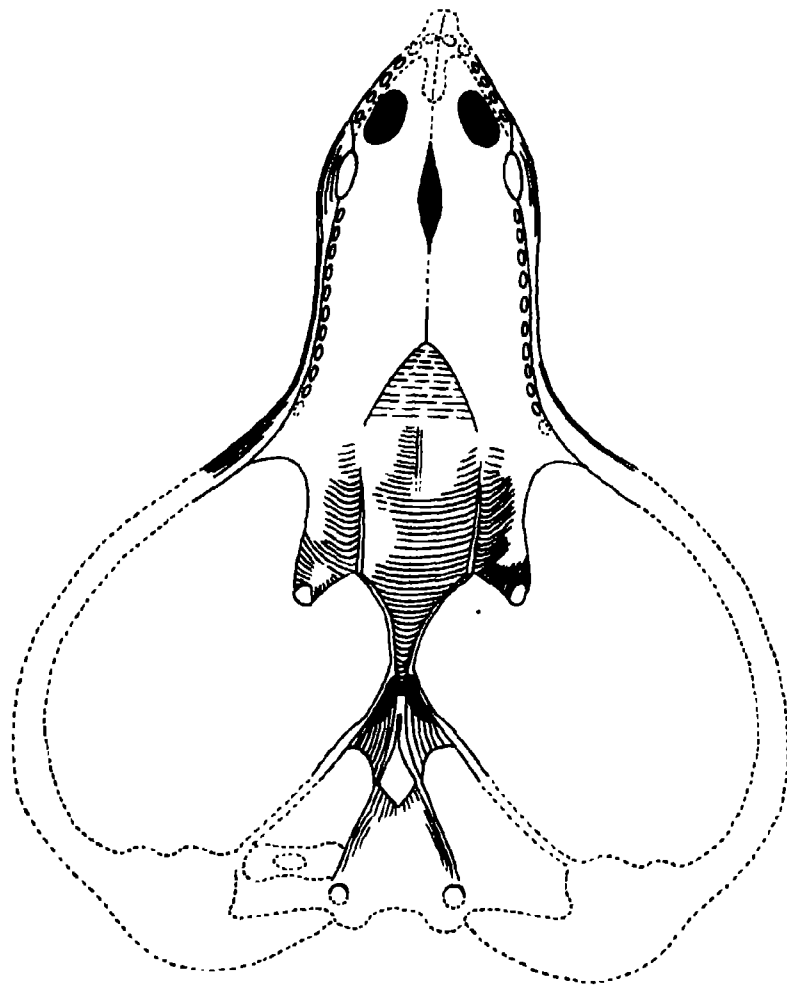


FIG. 8.—*Parathrinaxodon proops* gen. et spp. nov. Palate natural size.

The pterygoids have strong transverse processes and form the well-developed walls of the naso-pharyngeal passage which are continued posteriorly into the two vertical plates of the pterygoid bar. There is an inter-ptyergoid vacuity but posteriorly the pterygoids approach each other and finally meet in the region from which the quadrate rami are given off. This junction of the pterygoids, however, may be the result of the distortion of the right side of the skull. The quadrate ramus of the left pterygoid is missing and the right has been disturbed and its full extension cannot

be determined owing to the difficulty of cleaning such a thin strip of bone out of the hard, flinty matrix.

Sufficient of the left epipterygoid remains to show that it was flattened and similar to that of *Thrinaxodon*.

The basicranium is weathered and incomplete. Anteriorly there is a keel which passes forwards between the pterygoids and is continued backwards into a small triangular area which projects downwards from the main parasphenoid-basisphenoid complex, the posterior border of which, with the basioccipital, has been lost by weathering. The anterior border of the right foramen jugulare remains, however, to help to give the proportions of the skull.

On the left side the pleurosphenoid is visible fused to the front of the basisphenoid and rising upwards and outwards towards the epipterygoid.

Teeth—The teeth are, perhaps, the most interesting feature of the skull. The only incisor preserved is a replacing-tooth which is just erupting in the second of the preserved alveoli. The left canine is missing and the right badly damaged, but sufficient of the latter is preserved to give some indication of its size and to show that it was oval in shape.

In the left maxilla the first and third post-canine teeth are well preserved, the first being partly out of its socket, fig. 9. They are slightly compressed and spear shaped with a single cusp, the posterior border swelling out and having a crenulated edge running down towards the tip which in each tooth has been broken off. The second and fourth alveoli are empty but in the fifth is the tip of a replacing-tooth. The sixth tooth was fairly well preserved and the crown was removed for examination, fig. 9. In outer view is seen the remains of a large centre cusp with a small accessory cusp on either side. Internally there is a cingulum which carries a small accessory cusp anteriorly and apparently also a second accessory cusp posteriorly, but this latter was broken off and left in the matrix when the crown was removed.

There are the damaged remains of teeth in the eighth and tenth alveoli, but the seventh, ninth, and eleventh are empty. The eleven teeth occupied a length of 32 mm.

In the right maxilla the first four alveoli are empty. In the fifth, as in the left maxilla, is the tip of a replacing-tooth. The sixth post-canine is in place, but rather damaged, the seventh is missing, the eighth is present, but badly damaged, and the ninth just erupting. There are no teeth in the tenth or eleventh alveoli.

It seems quite plain that in this specimen the alternate post-canine teeth are being replaced in the manner found in *Thrinaxodon*; but that the replacement of the even numbered teeth had started in the front of the jaw before the replacement of the posterior odd numbered teeth had been completed. It might be argued that the absence of the first four post-canines in the right maxilla, and also the tenth, indicate that the loss of alternate teeth in the left maxilla and in the centre of the right was a post-mortem change. But the presence of replacing-teeth in both fifth alveoli and the right ninth alveolus, together with the presence of a replacing incisor, give fair evidence that the loss was due to replacement.

There are only three specimens of Cynodonts known from the Permian, *Cynosuchus suppostus*, OWEN, and *Cynosuchoides whaitsi* (HAUGHTON), from South Africa, and *Permocynodon sushkini*, SMITH WOODWARD, from Russia, and this new form at once recalls the latter. Unfortunately only a preliminary description of this has yet appeared (SUSHKIN, 1927) which, together with the incompleteness of the new form makes detailed comparison difficult. It is very similar, however, in the relatively small snout, the way in which the orbits face forwards, the dental formula and, in particular, the nature of the crowns of the post-canine teeth. It lacks, however, certain characters of the Russian form, in particular the perforation of the

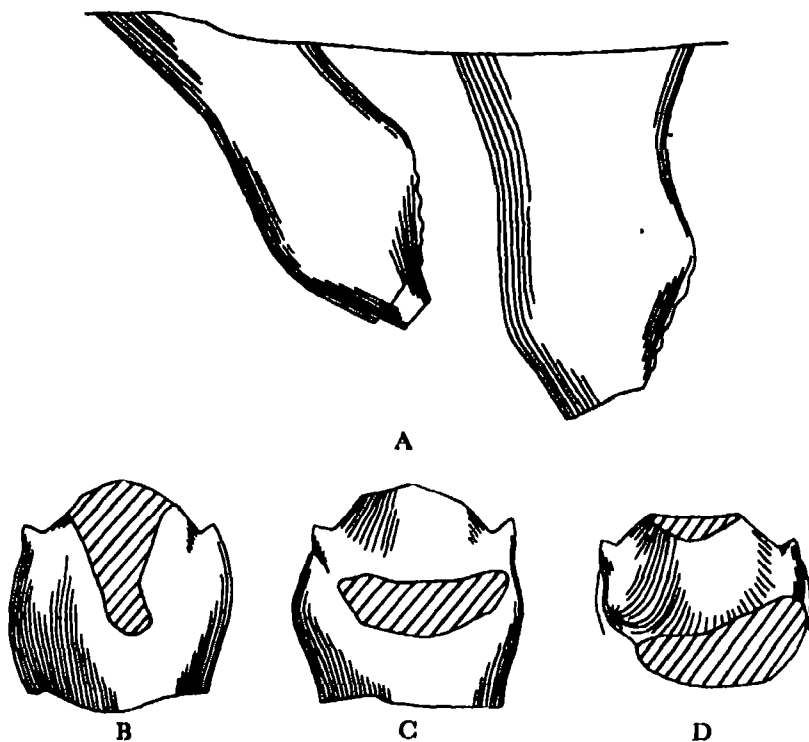


FIG. 9.—Teeth of *Parathrinaxodon proops* gen. et spp. nov. A, first and third left post-canines, outer view ; B, outer view of sixth left post-canine ; C, inner view of same ; D, same viewed from inner side and below. All x 10.

pits for the lower canines between the premaxillae and the maxillae, the constriction of the snout behind the canines, and the hooking of the premaxillae. In this form, too, the nasals are unusual in meeting the frontals well behind the front borders of the orbits.

Both forms are very similar to the Lower Triassic cynodont *Thrinaxodon liorhinus*, particularly in regard to the teeth. The possession of post-canine teeth having a large centre cusp with a small accessory cusp on either side in outer view, and two small accessory cusps lingually is now known in the two Upper Permian cynodonts, *Permocynodon sushkini* and *Parathrinaxodon proops*, the Lower Triassic forms *Thrinaxodon*

liorhinus and (probably) *Nyctosaurus larvatus*, and the Middle Triassic form *Tribolodon frerensis*. The presence, in the first two forms, of single cusped teeth anteriorly, and in *Thrinaxodon liorhinus* of teeth with only the three outer cusps suggests that these forms may eventually prove to be closely related.

The maxilla which BROOM (1930) described as *Cyrbasiodon boycei*, and suggested was possibly a Scaloposaurid Therocephalian, is probably a related form.

V--NOTE ON *Tribolodon frerensis*, SEELEY

The cynodont *Tribolodon frerensis*, SEELEY, has been known until recently only by the type specimen, an incomplete dentary with three teeth, collected from the Cynoganthus zone at Lady Frere, Cape Province.

Recently, however, BROILI and SCHRÖDER (1934) have described further material and they reached the conclusion that, as in the allied genera *Permocynodon* and

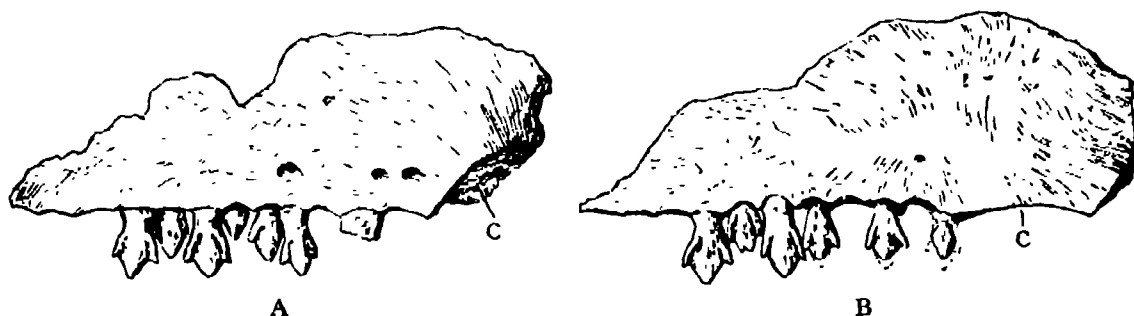


FIG. 10—Two maxillae of *Tribolodon frerensis*, SEELEY, to show replacement. $\times 2$. From BROILI and SCHRÖDER, 1934.

Thrinaxodon, it was impossible to distinguish premolars and molars as tooth-change could be observed throughout the post-canine series. It is plain from their illustrations of two maxillae, fig. 10, that here also the post-canine teeth were in two series the members of which were replaced at different stages. In one figure, A, six consecutive postcanine teeth are well preserved. The penultimate of the series is incompletely erupted, the next but one anteriorly shows only the centre cusp while the anterior tooth is loose in its socket and may have been on the point of being replaced. In the second maxilla, B, the replacement is not so clear, but it may be seen that there are four teeth of much the same size in alternate sockets, the first two being followed by empty alveoli, and the second two by larger teeth the first of which is apparently loose.

Though this evidence of alternate tooth-replacement is not by itself very clear yet taken in conjunction with the relationship of *Tribolodon* to *Parathrinaxodon* and *Thrinaxodon* it becomes fairly conclusive.

VI—NOTE ON *Dimetrodon*

When the nature of the tooth replacement found in *Thrinaxodon* was described to Professor D. M. S. WARSON he observed that he had seen similar replacement in some specimens of the Pelycosaur *Dimetrodon*, and very kindly placed at the author's disposal his collection of jaw fragments of this animal. In three of these the teeth are being replaced.

The first specimen, fig. 11A, consists of the anterior portion of a left mandible. The first and third teeth are in position though the crowns are damaged. In the second alveolus there is a replacing-tooth partly erupted, while the fourth alveolus, the last to be preserved, is apparently empty. This latter was ground down for about 3 mm and some small fragments were found which suggest that a damaged replacing-tooth may be present.

The second specimen, fig. 11B, is part of a left maxilla from the region in which this bone is swollen and carries the two enlarged canine-like teeth. Anteriorly

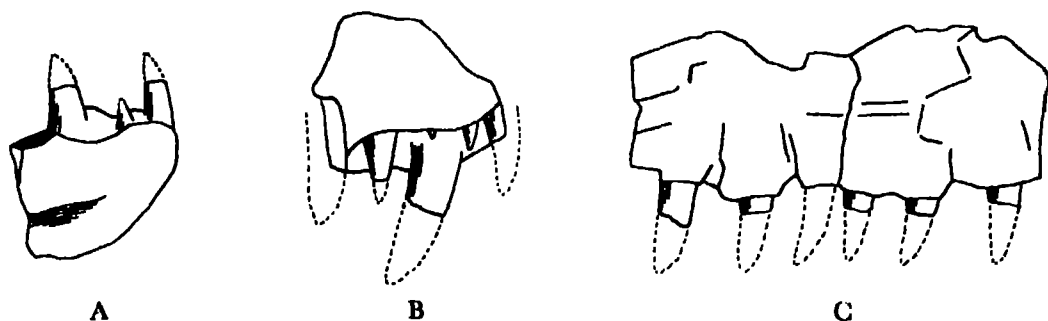


FIG. 11—Fragments of jaws of *Dimetrodon* spp. to show replacement of teeth. $\times \frac{1}{2}$.

there are the remains of the root of a large tooth which is apparently fully erupted. The second alveolus is occupied by a tooth which must have been just erupting at the death of the animal. The third tooth is very large, lacks the tip of the crown, and has at its base, and on the lingual side, the tip of a replacing-tooth showing. In the fourth alveolus there is a tooth which is incompletely erupted and this is followed by the remains of a root of a tooth which appears to have been fully developed.

The third specimen, fig. 11C, consists of some four inches of a right maxilla which has been badly fractured. The anterior (broken) border passed through the back of a tooth but there is not sufficient of this tooth preserved to show whether or not it was completely erupted. Remains of teeth are preserved in the second, fourth, and sixth, and the seventh, ninth, and eleventh alveoli. No teeth were found in the third and fifth, or the eighth, tenth, and twelfth alveoli (the first four of which have been cleaned of matrix and the last of which has been ground down some 4 mm), except possibly in the tenth where some small fragment, apparently of a tooth, may be the tip of a replacing-tooth.

These three specimens seem to indicate fairly clearly that, as in the described Cynodonts, the teeth in *Dimetrodon* belong in two series the members of which alternate and are replaced at somewhat different stages. If this is true, there are three points which call for some comment.

In the specimen B the first, third, and fifth teeth are well developed while the second and fourth are being replaced. There is, however, a replacing-tooth at the base of the third. The fact that this tooth is only just appearing while the second and fourth replacing-teeth are partly erupted suggests that the replacement of the, in this specimen, odd numbered teeth must have followed quickly on the replacement of the even numbered.

In the third specimen the most outstanding feature is the apparent absence of replacing-teeth from the empty alveoli, a condition which might be claimed to show that the loss of certain teeth was purely fortuitous and had nothing to do with replacement. We know, however, that during the replacement of a tooth the bone is resorbed around the roots and that the replacing-tooth moves down into an enlarged alveolus. In any animal which died during this process there would be a greater chance of the loss of a replacing-tooth than of those teeth which are held in the jaw in a normal manner.

It is also necessary to explain the fact that it is the odd numbered teeth which are being replaced in the anterior region, while the even numbered are being replaced in the posterior region. If, as seems to be indicated by the second specimen, the replacement of the second series follows very shortly after the first series, it is not unreasonable to suppose that the replacement of the first series (in this specimen the even numbered teeth) may not have been completed throughout the jaw before the replacement of the alternate series commenced.

VII—DISCUSSION

The evidence on tooth replacement afforded by the material described may be summarized as follows :—

- (1) In the cynodont *Thrinaxodon liorhinus* the first incisors and the canine teeth are replaced while the animal is young.
- (2) In the cynodonts *Parathrinaxodon proops*, *Thrinaxodon liorhinus*, and *Tribolodon frerensis* the post-canine teeth belong to two series. The members are situated alternately in the jaw and are replaced at different stages. There is evidence of only one replacement of each tooth.
- (3) The replacement of alternate teeth at different stages seems to have been present in the most primitive of the mammal-like reptiles (the Pelycosaur) as is shown by three pieces of jaws of *Dimetrodon*.

That this "distichical" condition is typical of the non-mammal-like reptiles has already been shown by BOLK (1922). BOLK called attention to the fact that the tooth germs of living reptiles are situated alternately on the buccal side and at the

base of the dental lamina, and called the two types "parietal" and "terminal". The row of parietal tooth germs he called the "exostichos", the row of terminal germs the "endostichos". He showed that the teeth of the exostichos always appeared in an embryo before those of the endostichos, but that they were not replaced by the latter, the teeth of the two rows being functional at the same time, each being replaced by further members of its own "family".

It is of interest at this point to consider the condition found in the earlier vertebrates. There is nothing known about the tooth-replacement in the Cotylosaurs, among which forms the common ancestors to the mammal-like and "true" reptiles may be sought. But for the forms which may be considered to be ancestral to all reptiles, the early amphibia, we have the work of Professor D. M. S. WATSON.

In his description of the teeth of the Carboniferous Labyrinthodonts (the Embolomeri) WATSON (1926) says "They are attached to their supporting bones by fusion, and are shed by resorption of the bone. When so lost they are replaced by a new tooth, which has been developing in a neighbouring emplacement. The whole dentition thus consists of a series of pairs of teeth which are functional alternately." Such a condition might be expected to occur in an animal in which the teeth were distichic if the development of the replacing-tooth was a very slow process, or if the life of a tooth was very short. The very great similarity between the dentitions of the primitive Crossopterygian fishes (the Osteolepids) and the Labyrinthodonts, in regard to both the structure of the teeth and the presence of large tusks by the side of which are pits for replacing-teeth (*idem*), make it almost certain that the Osteolepids also had the teeth in two alternating series.

It seems, therefore, that distichical arrangement of the teeth was inherited by both mammal-like reptiles and true reptiles from the earliest tetrapods and the primitive bony fishes. This conclusion supports BOLK's suggestion that the condition is inherited from very primitive ancestors and may be seen in the arrangement of the teeth in modern sharks.

The question of the origin of the mammalian type of tooth-replacement may now be considered.

BOLK claimed that the milk and permanent dentitions of mammals do not represent two surviving members of each reptilian tooth "family", as was commonly believed, but that the milk teeth are the sole surviving members of the exostichos while the permanent teeth represent the survivors of the endostichos. This condition, in which the members of the two rows were not functional at the same time, BOLK called "chorisstichic," and believed it to be the result of an increase in the time interval between the eruption of the two rows. In support of the view he pointed out that the time interval between the eruption of the two sets differed very greatly among various reptiles; and that in the mammals, while the interval is very considerable, the germs of both sets are formed very early.

The establishment of the distichical condition in the post-canines of the described cynodonts, together with the fact that there is a very considerable interval in time between the replacement of the two sets in *Thrinaxodon*, adds some confirmation to

BOLK's views. In the maxillae, at least, there appear to be only two members of each tooth family left in place of the large numbers typical of reptiles. Carried one stage further this reduction would mean that such an animal would erupt one set of teeth and then, later, another set between the members of the first. If at this stage the teeth became crowded, possibly by increase in size and complexity, the second set might well replace the first instead of erupting between them.

It is still necessary to account for the apparent double replacement in the incisors in *Thrinaxodon*. While this might well be explained by the retention of an extra member of each "family", it should also be remembered that the "reptilian" method of replacement, when the replacing-tooth appears from below and inside the older tooth, is only possible in a tooth in which the pulp cavity has remained widely open. The development of incisor teeth in which the root is nearly closed, as in *Thrinaxodon*, would mean that successive members of each tooth "family" would have to erupt at the side of their predecessors, a condition observed in this genus and also in *Galesaurus planiceps*, OWEN (PARRINGTON, 1934). It is thus impossible to distinguish members of the exostichos and endostichos in the incisors by the manner of their eruption. Now, while the first upper incisor is being replaced in the very young *Thrinaxodon*, specimen B, it is the second which is apparently erupting in the adult, specimen H, and this apparent double replacement in the incisors may well be due to a very considerable interval between the replacement of the exostichos, of which row the first incisor would be a member, and of the endostichos which would include the second incisor.

It is of interest at this point to consider the question of tooth replacement in the remaining Triassic Theriodonts.

The majority of the remaining Cynodonts have a gomphodont dentition (*Diademodon*, *Trirachodon*, etc.) and, as has already been pointed out, evidence of actual replacement in these forms is very rare. It is certain, however, that the teeth were subject to considerable wear, probably by an antero-posterior movement of the lower jaw. If the alternate teeth had been replaced at different times in the manner of *Thrinaxodon*, it would seem likely that some specimens would have been found by now which would have demonstrated this distichical condition by the different state of wear of alternate teeth. The absence of such specimens, and the occurrence of specimens which show a steadily progressive decrease in the amount of wear of the teeth towards the back of the jaw, seem to indicate that in these forms the mammalian chorisstichic condition had been reached, possibly as the result of the relatively large size of the teeth.

WATSON (1931) has shown that in the early Bauriamorph *Eriolacerta parva* the replacement is of the reptilian type, because damage to the crown of one of the cheek teeth exposed a successor immediately below it. He has also showed that there is reason to suppose that the whole of the Bauriamorpha were the descendants of the Scolaposaurid Therocephalia. However, while the condition in *Eriolacerta* may have been typical of the group, a more advanced condition could have been present in the gomphodont forms as in the gomphodont cynodonts.

A further, and very important problem, on which some light has been thrown, is that of the possibly compound nature of mammalian teeth.

BOLK studied the structure of the enamel organs of mammalian and reptilian teeth, and came to the conclusion that the enamel organs of mammalian teeth are compound. He reasoned—against the weight of palaeontological evidence—that the teeth were therefore also compound, each being built up of two elements homologous with a reptilian tooth. The fact that in the early cynodonts there is a true reptilian succession of complex teeth seems evidence against such a view. But it is still possible to argue that the fusion of pairs of members of each family of reptilian teeth could have taken place at an earlier stage, and thus these reptiles also possessed “dimer” teeth.

I am greatly indebted to Dr. W. D. LANG and Dr. W. E. SWINTON of the British Museum (Natural History) for the loan of the three specimens of *Thrinaxodon liorhinus* in their charge, and to Professor D. M. S. WATSON for the generous loan of his *Dimetrodon* material and advice on the question of the restorations of *Nyctosaurus* and *Thrinaxodon*. And finally I would like to take this opportunity of thanking the Balfour Managers for their sympathetic interest during my tenure of the Studentship, particularly in regard to my expedition to the Ruhuhu Valley in Tanganyika.

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V—Studies on the Epidermal Structures of Birds

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(Communicated by A. S. PARKES, F.R.S. —Received April 30—Revised November 7, 1935—Read February 13, 1936)

[PLATES 16-28]

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I—THE DEVELOPMENT OF FEATHERS

I—INTRODUCTION

Studies on the development of feathers have as yet provided only inadequate answers to many fundamental problems. The evolution of feathers themselves and their relation to reptilian scales are still matters of hypothesis, and will remain so until more crucial information is forthcoming. Again, the phylogenetic sequence of events relating embryonic to definitive feathers is inconclusive, and it cannot be said with certainty whether the embryonic down feather is a secondary modification of the ordinary contour feather, or whether the latter has been evolved from a primitive down-like primary feather.

Investigations upon the development of epidermal structures in general, and of feathers in particular, were favoured by workers in Germany during the last century, culminating in the classical work of DAVIES (1889) on the development of feathers in the pigeon. His views still form the accepted basis for work on plumage, but recent knowledge has necessitated a re-examination of them. (LILLIE and JUHN, 1932.)

In 1902 STRONG wrote an admirable account of the definitive feather in *Sterna hirundo*, which agrees fundamentally with the conclusions reached by DAVIES, although reference is made only to the latter for the development of the embryo feather.

COSSAR EWART's work on the nestling feathers of the Mallard (1921) was followed by LAMONT's study of the development of embryo feathers in the Indian Runner duckling (1925). As the former dealt with external characters, and the latter took stages at intervals of several days and rarely sectioned them, knowledge concerning the structure and development of the embryo feather has advanced little since DAVIES's time.

Modern work on plumage reactions in relation to internal secretions has emphasized the need for a reconsideration of feather development, if satisfactory theories are to be formulated to explain the accumulated experimental data.

In this paper the sequence of plumage has been observed in several types of birds, and the histological development has been followed from the embryo to the adult.

II—MATERIAL AND METHODS

(a) Embryology of Nestling Feathers

For this study, stages were taken at daily intervals during the incubation period of the Domestic Fowl and two varieties of duckling (Khaki Campbell and Aylesbury). The Chinese Gosling was also used, but owing to poor fertility only four stages were obtained. These, however, were at convenient intervals for comparison.

The embryos were fixed in 10% formalin, picro-nitric or Bouin's solution. The latter, proving the most successful fixative was the most generally used, and was always heated to incubator temperature before immersion of the embryo.

(b) Definitive Feathers

Two varieties of the domestic fowl (Rhode Island Red, and the offspring of a Black Leghorn X Light Sussex) were reared in a bachelor brooder and watched for changes in plumage. At the age of eight weeks they were transferred to folding units out of doors. Stages were taken at intervals of two weeks in the former, and weekly in the latter over a period of eighteen weeks.

In addition, a series of Khaki Campbell ducklings (from one to eight weeks old) and a series of nestling starlings were obtained for comparison.

The skins of these birds were fixed in Bouin's solution after representative feathers had been plucked for reference.

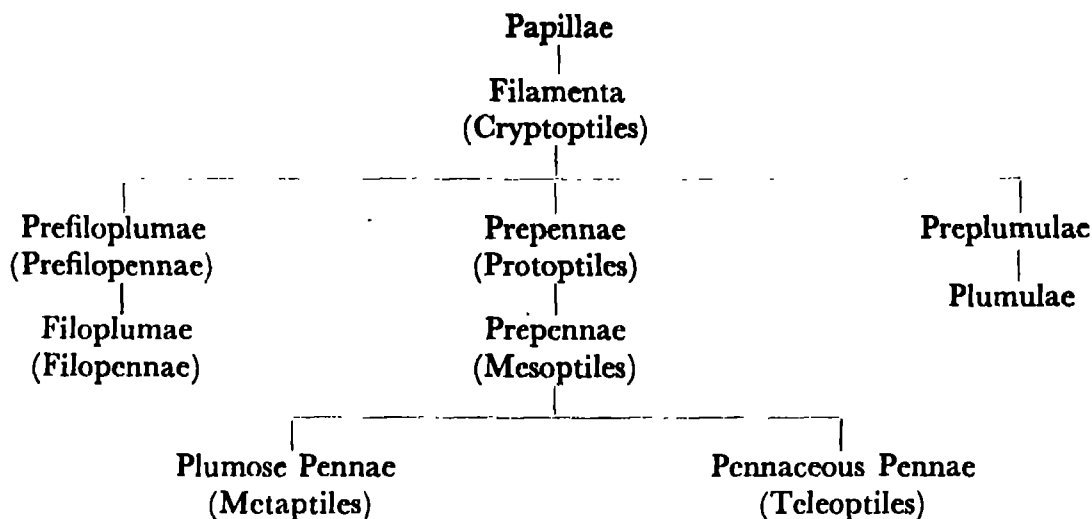
Portions of the skin were embedded in celloidin followed by paraffin wax. It was found necessary to embed the embryo feathers for two weeks in celloidin and the definitive feathers for at least three weeks, to get good infiltration. In the next stage, the small feathers were embedded rapidly in wax in the usual way, but larger feathers were left in a mixture of chloroform and wax for 24 hours at 40° C before embedding rapidly at a higher temperature.

Sections were cut from 6-8 μ in thickness, and stained by the short method of iron haematoxylin (*i.e.*, only 15 minutes in both mordant and stain) otherwise it was impossible to differentiate the cornified parts successfully. Orange G, picro-indigo-carmin or picro-fuchsin were used as counter stains. In older feathers the last-named gave the most pleasing effect.

Whole feathers from the different pterylae were always mounted dry for comparison.

III—THE SEQUENCE OF PLUMAGE IN BIRDS

It will be as well at the outset to ensure a correct interpretation of terms used in this work, by reference to the table compiled by EWART (1921), showing the sequence of feathers in a hypothetical Avian type from embryo to adult.



Prefiloplumae, preplumulae and prepennae are typical constituents of nestling down, and will therefore be collectively spoken of as *Neossoptiles*; and instead of restricting the term *Teleoptiles* to Pennaceous Pennae as in the above table, it will be used for all types of adult feathers, as originally used by GADOW (1907), PYCRAFT (1907) and NEWTON (1896).

Hence the above table can be modified to give a clearer representation of what is usually found in feather sequence from the embryo to the adult bird.

Papillae

Filamenta

Neossoptiles (nestling feathers, consisting of prepennae, prefiloplumae and preplumulae).

Two generations are known :

- (a) Protoptiles—prepennae, prefiloplumae and preplumulae.
- (b) Mesoptiles—as yet known to be derived from prepennae only, or to arise directly.

Teleoptiles (adult feathers).

Three types exist :

- (a) Plumulae—*i.e.*, down feathers.
- (b) Filoplumae.
- (c) Pennae—*i.e.*, quill feathers, whether plumose or pennaceous.

Of all the types of fledglings examined in the present investigation, only in the duck are three types of neossoptiles developed. Prepennae and preplumulae are present in the goose, and prepennae only in the fowl, on hatching. In birds with nidicolous young (*e.g.*, starlings) prepennae are present, but sparse and restricted in area.

Fowls—For reasons to be discussed later (*see* p. 169), it is assumed that the coat worn by the newly-hatched chick consists of protoptiles, the mesoptiles having been suppressed.

While it must be remembered that great variability exists between members of a flock, the order of appearance of feathers in the different pterylae is invariably constant, the difference being one of time only. On hatching, all except the most proximal primary and secondary flight feathers can usually be seen, together with the tectrices majores. These teleoptiles all bear protoptiles at their tips, which are shed during the first week after hatching. The remaining wing feathers (tectrices media and minores) and some body feathers in each area usually appear during the first month. The latter develop in the following order :—thigh, breast and neck, head, back, abdomen. In the Rhode Island Red fowl, tail teleoptiles are found in females during the second week, but the tail of the male is retarded in development. At 6 weeks, some teleoptiles are present in each pteryla, and the wings are completely fledged. Few bear protoptiles although many are still present between the teleoptiles, the latter being replaced completely by the end of the eighth week.

From the age of eight weeks to four months, the feathers are gradually shed from all pterylae and replaced by typical adult feathers with mature sexual characteristics.

After the assumption of this final type of plumage, which can only be changed by an improperly balanced endocrine condition (TORREY and HORNING 1925), the gradual moulting ceases, and the feathers replacing those shed in the annual moult are similar in every respect to their predecessors, except under pathological conditions.

Ducklings—The main difference between the method of adoption of the teleoptile coat in the fowl and the duck appears to be correlated with differences in the habits of the birds. In the former, where the wings are of greater use than the tail, the first generation of feathers is replaced by the teleoptiles at an early age. In ducklings, however, where the wings are of little use, they are greatly retarded in development, and teleoptiles do not appear until the fifth week after hatching. The tail, which is of some advantage in swimming, shows enormous elongation of the calamus of the protoptiles during the first and second weeks after hatching. These are borne upon the tips of the teleoptiles, which appear during the fourth week, until the sixth week. On the rest of the body teleoptiles appear on the back, legs, and head during the third week, and in other pterylae about the sixth week. By the seventh week they are widespread, and most protoptiles have been shed.

The remiges, after their retarded start, grow very rapidly and during the eighth week surpass the greater wing coverts in size. No trace of the mesoptiles found in the ninth pair of rectrices of the Mallard duck (EWART 1921) have so far been found in the Khaki Campbell duckling.

Thus the neossoptiles present in all nidifugous birds (*i.e.*, young completely clothed with nestling down) and to a lesser extent in nidicolous young, are replaced by teleoptiles during the nestling period. The order of formation of the adult

feathers differs in birds of different habits, *e.g.*, Galliformes and Passeriformes show precocious development of remiges and Anseriformes of rectrices. On the rest of the body, teleoptiles appear in the following order : thigh, breast and neck, head, back, abdomen.

IV—NESTLING FEATHERS

(a) *A Comparison of Neossoptiles and Plumulae*

The development of the neossoptiles in the duck, goose, and fowl is more easily understood after a comparison of the external features of the various types of down.

Feathers first appear externally as minute opaque spots on the otherwise transparent skin of the spinal and femoral tracts of the embryo on the seventh day in the chick, the ninth day in the duckling, and about the twelfth day in the gosling. It was impossible to gauge the exact age of the Passerine embryos studied, but the protoptiles appear in the different pterylae in the same order as in the nidifugous types. The feather papillae are arranged in diagonal rows, in such a manner that some three days later, the backwardly growing rudiments overlap, as do reptilian scales. Feathers appear on the rest of the body a few days after their appearance on the back, but their development on the wings is delayed until the tenth and thirteenth days in the chick and duckling respectively.

At about this time, small papillae may be seen forming a circle round the base of each larger papilla in the duckling and gosling, but absent in the chick. These are the preplumulae from whose follicles the plumules of the next generation of feathers will be developed. The larger feather filaments are the prepennae. In the duckling the preplumulae may be secondarily divided on account of their size. The smaller papillae, which appear last, are the predecessors of the filoplumes.

On hatching, the young birds are clothed in long backwardly projecting filaments, all of which have reached the same state of development in spite of their different times of appearance. In a few hours the filaments are dry, and then if friction, however slight, is applied, the sheath splits and the barbs splay out forming a dense covering of soft, warm down.

In the duckling, various types of down may be distinguished, differing in size and texture, but having the same general plan. According to NEWTON (1896) the neossoptiles of Anseres have a "feeble rhachis" bearing "all the biserially radiated rami, forming feathers, which clearly resemble the down of mature birds, and are devoid of an aftershaft". In reality, the prepennae of a duckling consist of both shaft and aftershaft, which are comparatively well developed, fig. 28, Plate 19. These unite near the base forming a definite calamus. Barbs, or rami, are arranged biserially as NEWTON says, but along both rhachis and hyporhachis, the former ending in two long barbs, which are characteristic of the duckling. The prepennae of the head are small, and with no caps in the short calamus. On the breast and back they are much longer, and usually have at least one cap on hatching ; while the tail prepennae are very long and stiff, with bristly barbs and a long calamus

containing several caps. The barbules are also arranged biserially along each barb of both shaft and aftershaft, but a superficial spiral arrangement is obtained by the twisting of the proximal cell of each barbule.

The adult down of a duck is fundamentally similar to the nestling down. Numerous slender barbs, only slightly longer than the barbs of the prepennae but much longer than those of the preplumulae, join forming a long rhachis in the shaft; while the aftershaft consists of fewer barbs, which are equal in length to those of the main shaft, and which join to form a definite hyporhachis. The chief structural difference lies in the restriction of cilia to the distal part of the barbule, while the next two or three cells bear pyramidal swellings, and the proximal cells are uniformly elongated with no visible swellings at the nodes, fig. 29*e*, Plate 20.

The neossoptiles of a newly-hatched gosling are essentially similar to those of a duckling. The rhachis is shorter in the gosling, ending in two very long, slender barbs, and the cilia from the nodes of the barbules are much longer than in the duckling, especially the distal cilia, fig. 29*e*, Plate 20.

The adult down of the goose consists of a veritable forest of barbs, but structurally differs from the nestling down only in the presence of pyramidal swellings between the elongated basal cells of the barbule and the ciliated distal cells, fig. 29*f*, Plate 20. This condition is very similar to that of the duckling, fig. 29*e*, Plate 20.

The neossoptiles of the chick, fig. 27, Plate 19, present a totally different appearance from those of the duckling and gosling. The rhachis is here so short that from a cursory glance the feather appears umbelliform. Most writers mention one barb being longer and stronger than the rest, and consequently regard it as a rhachis. But all the barbs are approximately of equal length, and the short-rhachis is formed by the fusion of two of these, while others join lower down. The aftershaft is well developed, as in ducklings, consisting of barbs almost as long as the shaft; but the calamus is very short and contains no caps. The barbules are not twisted, but the basal cell is very broad and each subsequent cell is swollen at the node in receiving the narrow proximal end of the next cell; and no cilia are present, fig. 29*a*, Plate 20.

Unlike the duckling, there is no distinction except in size between neossoptiles from any part of the body, the tail feathers being as soft and slight as those on the breast.

A few days before hatching, the filaments of the protoptiles of the remiges are pushed out from their follicles by the rapidly growing teleoptiles, fig. 27, Plate 19. This, as will be discussed later, is due to continuity between a few barbs of the teleoptile and the calamus of the protoptile. The teleoptile consists of a very definite rhachis, which branches alternately at close intervals. The branch barbs are progressively shorter from the base of the feather upwards, so that they all end at the same level. Near the base about four pairs of shorter barbs representing the aftershaft, with no definite rhachis, join the shaft, forming a short calamus. Each barb bears biserially arranged barbules extending along its whole length, thus no long slender filaments form the termination of the barb as in protoptiles. The single barbule of a teleoptile represented in fig. 29*b*, Plate 20, shows great specialization

compared with the protoptile barbule in fig. 29*a*. The basal cell is still swollen, but its distal end bears a curved projection. This is emphasized in the next two or three cells, where the projection forms a typical barbicel. Thus a slight amount of interlocking is possible in the tip of the teleoptile.

The adult down of the fowl differs from the nestling down in having a relatively longer rhachis, so that the feather loses the umbelliform appearance of its predecessor.

The plumule of the fowl is really comparable to the preplumule, which is not developed in the chick, but as already shown for the duckling, the preplumule is only a minute edition of the prepenna. It is not comparable to the teleoptile described on page 149, which is here classed among nestling feathers, as it is developed during the incubation period, but the structure of which forms the fundamental basis of all teleoptiles, whether contour or quill feathers.

(b) The Development of Protoptiles

In early stages of development, the epidermis consists of two layers, an upper epitrichium with elongated nuclei, and a lower or inner stratum germinativum (stratum Malpighii or rete mucosum) consisting of cells with rounded nuclei. The latter layer is by far the most important and ultimately gives rise to all the layers present in the adult skin, and to the derivatives of the epidermis such as scales or feathers.

By continued proliferation, the stratum Malpighii gives rise to several layers, the lowermost of which has typically cylindrical nuclei and hence bears the name of stratum cylindricum. Above this are the numerous rounded cells of the stratum intermedium which effectually cut off the epitrichium from nourishment, so that it atrophies, becoming replaced by the stratum corneum.

All the layers of the epidermis of the adult bird therefore are directly derived from the lower of the two layers found in the embryo.

(i) Ducklings

The development of protoptiles in the duck has been studied by comparing stages during the incubation period at intervals of one day, starting from the eighth day.

8 days—The site of the feather embryo is visible microscopically in the spinal and femoral tracts. At intervals in transverse sections of the skin from these parts, a few of the rounded nuclei of the Malpighian layer appear slightly elongated, with their long axes vertical, *i.e.*, at right angles to the axes of the nuclei of the epitrichial layer.

9 days—The feather germs may be seen macroscopically as small white patches in the spinal and femoral tracts. The elongation of cells of the stratum Malpighii is even more apparent, and certain cells of the epitrichial layer have divided, the daughter cells passing down to the position of the future intermediate cells.

This migration of epitrichial cells is interesting, as according to DAVIES (1889), the epitrichium takes but a passive part in feather development, remaining as a very thin covering external to the sheath, and soon being stretched to the breaking point.

10 days—The feather germs are visible macroscopically over the whole body. In an Aylesbury duckling of the tenth day, the whole of the feather germ is slightly depressed, fig. 1, Plate 16, as in early stages of hair development, but in the Khaki Campbell duckling it is correspondingly elevated. The epitrichial layer no longer contains a single row of nuclei, but two less regular rows, which are distinct from the nuclei of the intermediate cells (formed by the rapid division of the Malpighian layer cells) in the direction of their long axes.

11 days—The feather germ is distinctly elevated, and has increased to more than twice the size on the previous day, as may be seen by comparing figs. 1 and 3. The epitrichial layer is less compact than formerly, and is obviously dividing less rapidly than the Malpighian layer, which is now many cells wide. On one side of the feather germ, the intermediate cells are much less compact, and at that side the slope is more gradual, fig. 2, Plate 16. DAVIES (1889) attributes this to more active division at the steep sloped (*i.e.*, the anterior) side of the feather, resulting in a bending backwards.

A clear space between the dermis and Malpighian layer, which persists throughout the development of the feather, is possibly analogous with the "basal membrane" noted by STRONG (1902a). High power microscopic examination reveals this to be composed of the bases of cells of the stratum Malpighii whose nuclei are uniformly withdrawn toward the periphery, and so agrees with the observation of JEFFRIES (1883).

Below the concentrated dermis of the feather germ are always two or more blood vessels. A distinct sheath envelops the feather before the next day of incubation, which according to LAMONT (1925) is one cell deep and composed of flattened cells "derived from the epitrichium or outer layer of the epidermis". In both Khaki Campbell and Aylesbury ducklings, however, the sheath may be three or more layers wide, consisting of the epitrichial layer and its derivatives together with some intermediate cells, which have become secondarily elongated in the same direction as the cells of the epitrichial layer. This agrees with DAVIES's observations on the pigeon (1889).

The innermost layer of intermediate cells has now definite elongated nuclei, and unlike DAVIES's description of the pigeon, this shape is more or less retained until the final stages of development.

12—13 days—A tendency for the intermediate cells to become arranged in groups is apparent, at least in the Aylesbury duckling, although this process is delayed until the following day in the Khaki Campbell duckling.

14 days—Longitudinal sections on this day show that the base of the feather germ is slightly sunken below the general level of the skin. This is due to a downgrowth of the epidermis of the feather papilla and the surrounding skin, forming the feather follicle. The pulp in this region is very concentrated, and as the follicle becomes

deeper, this dense pulp moves downwards so that it is always situated near the base of the feather, *i.e.*, the growing region. The blood supply consists of a central vessel surrounded by a circle of smaller vessels, but this is more clearly defined in later stages of development.

The grouping of intermediate cells, forming the so-called ridges seen in transverse sections on the 12th and 13th days of incubation, is complete. The stratum cylindricum has passed outwards between the ridges, eventually meeting the sheath cells, and thus continuing to form a boundary between the pulp and intermediate cells. According to DAVIES, the cylinder cells may extend between the sheath and intermediate cells of each ridge, but this certainly does not occur in the duck, goose or fowl.

The ridges are of unequal size, the larger ones tending to be concentrated to one side of the feather embryo, fig. 6, Plate 16, and in the largest of these in the Khaki Campbell duckling, pigment cells are present. Fig. 5, Plate 16, shows the ridges between the lines *a* and *b* in fig. 6 highly magnified, and the detailed structure of the pigment cells is clear.

15 days—During the late 14th and 15th days of incubation, the intermediate cells of each ridge become separated into three groups, the so-called “median” and “lateral plates”. The median plate consists of small, rounded cells, which remain scattered about the central part of each ridge, figs. 2 and 8, Plates 16 and 17, until the 16–17th days, when they withdraw towards the apex of the ridge, forming a dense mass—the future barb, fig. 9, Plate 17. The lateral plates undergo a more rapid development. During the 14th day, in transverse section they appear to consist of a string of flattened cells on either side of the median plate, but each cell is really quite separate from its neighbour. This becomes emphasized as development proceeds, until it is clear that each is a cross-section of a developing barbule. Fig. 29*d*, Plate 20, shows the single barbule from a newly hatched duckling, and the longitudinal section in fig. 8 illustrates how the fundamental structure of the barbule (a single row of cells placed end to end) is laid down as early as the 15th day of incubation.

16 days—The feather follicles are comparatively deep, fig. 7, Plate 17, and horizontal sections show that each is at the “knot of a mesh of muscle network”, which LAMONT (1925) also noticed in a 17 days embryo Indian Runner duckling. At the distal end of a feather embryo about this time, signs of approaching cornification are to be seen in the shrinkage of cylinder cells from between adjacent ridges. This is foreshadowed in Aylesbury ducklings of the 15th day, in which as fig. 8 shows, the separation of barbules from barbs and of cylinder cells from barbules has commenced.

17 days—During the 16th and 17th days the barbs are formed by cells of the median plate in each ridge moving inwards towards the apex. Hence many newly formed barbs are pear-shaped in cross-section, with the pointed end consisting of traces of the last cells incorporated into the barb. Many authors mention “residual cells”, which occupy spaces in the ridges, but these are very rare in the Khaki Campbell and Aylesbury ducklings.

18 days—The pulp of the distal end is now composed of very scattered nuclei connected by cytoplasmic strands, with a few blood vessels, and bounded by the cylinder cell layer which differs greatly from its appearance in earlier stages, fig. 9. The once distinct layer of compact cylindrical cells now forms a nucleated string with no definitely shaped cells, which is, as STRONG suggests, probably due to the great longitudinal growth of barbs and barbules. The barbs are differentiated into a central medulla, consisting of large vacuolated cells, and a thick-walled cortex of quadrangular cells with densely-staining properties. One barb is relatively large, with progressively smaller barbs on either side and very small barbs opposite. As these very small barbs are absent from the tip of the feather, they must represent the distal ends of shorter barbs. All pigment cells proper have disappeared before the barbs are formed, *i.e.*, the pigment granules have been distributed to the outermost cells of the cortex and all the barbule cells. Presumably the amoeboid processes of the original pigment cells are withdrawn, when their function is completed, and the cell which remains behaves as, and is indistinguishable from, an ordinary intermediate cell. The barbules are attached to the barbs near their apices, by cells elongated radially instead of lengthwise as are the barbule cells distally. This results in the flattened bases of the barbules seen in fig. 29, Plate 20.

The sheath is partly cornified, having the characteristic "layered" appearance, and no distinct cells being visible, but very occasional nuclei. The outermost epitrichial layers are now quite indistinct, being broken at intervals as early as the 15th day of incubation, and with long spaces between nuclei.

19 days—Preparations for cornification have proceeded still further, in that the cylinder cell layer has withdrawn from between ridges, and now forms a more or less circular boundary of the pulp, in transverse section. On this day, too, are the first signs of branching of the largest barb seen. This strongest barb is regarded as the rhachis.

20 days—The central cells of the rhachis appear greatly enlarged and have begun to become vacuolated, the comparatively small nuclei remaining attached by cytoplasm to the cell wall for some time. The smaller barbs become medullated soon after the rhachis and in a similar manner. The rhachis frequently branches, as more rarely do some of the small barbs. This "branching" is really fusion of barbs, for, as the feather grows from the base, the tips of the barbs are formed first. The cortical cells nearest the periphery are the first to fuse, the rest of the barb remaining quite distinct for some time, and after complete fusion, the cortical cells remain separating the medulla into two parts for a considerable distance downwards. From this method of fusion it would thus be impossible for a feather to have a spiral arrangement of barbs; and the method of attachment of the barbules permits of no other than lateral arrangement of barbules, although spiral arrangement is superficially obtained in the nestling and adult down of Anseriformes by the twisting of the basal cells, figs. 29c-f, Plate 20.

21 days—Fusion of barbs occurs very rapidly in later stages of development, culminating in the calamus about the 24th day. Transverse sections near the base

of an embryo feather on the 21st day show the outer cortical cells of the rhachis extending almost completely round the pulp, which is very dense in this region, and which is separated from the intermediate cells by clearly defined cylindrical cells. The intermediate cells are arranged in two groups on the inside of the thickest part of the rhachis. This is seen in succeeding sections to be due to the recent fusion of two branch barbs. The whole of the tip of the feather is cornified, while only the peripheral cortical cells near the base show signs of approaching cornification. This process always commences by the coalescence of cells radially, so that the elongated nuclei seem to lie in a "layered" material. In later stages, the layered appearance is seen to precede the complete coalescence of cells, so that the nuclei now lie in a uniform matrix. Meanwhile the cell walls have become remarkably thick, literally squeezing the nuclei out of existence, so that their position is represented only by a short line (probably a minute cavity) when cornification is complete. In the vacuolated medullary cells this cavity is relatively large, as perhaps owing to vacuolation taking place comparatively early in development, the cell walls are only slightly thickened; but no line of demarcation separates them from the cortical cells. LAMONT's definition of cornification as "loss of cellular structure" is thus singularly applicable.

Meanwhile the feather follicle has become gradually deeper, owing to the continued downgrowth of the epidermis. Continuity is clearly seen between the stratum Malpighii of the follicle and of the base of the feather; but the epitrichial layers of both feather and follicle are indistinct. This is due to the proliferation of the latter layers giving rise to a continuous sheath round the base of the feather, but higher up, the narrow follicle sheath is separate from the broader feather sheath. As the epidermis of the follicle does not divide into intermediate cells, and yet a definite sheath is formed, this seems to be evidence in favour of the theory that the epitrichium is largely responsible for that structure in embryo feathers.

Transverse sections show the deepest part of the follicle to be in the form of a double crescent of epidermal cells, with the convexity nearest the surface of the skin, including the combined sheaths of the feather and its follicle. Below this, the characteristic whorls of dermal nuclei with very small blood vessels are seen, and the dermis surrounding the whole of the follicle has a similar whorled appearance.

22-24 days—Sections from the base of the feather show the gradual fusion of large barbs with the rhachis on the upper side of the feather germ (*i.e.*, the side nearest the upper surface of the skin, when the feather is in its follicle). The rapid fusion of the smaller barbs on the opposite side forming the short hyporhachis, terminates in the lateral fusion of the shaft and aftershaft, giving rise to a short calamus, fig. 19, Plate 18. Cornification takes place very slowly at points of fusion, fig. 20, Plate 18, for there the cortex is always deeply stained instead of clear as when completely cornified.

Figs. 17-21, Plate 18, are transverse sections taken at different levels of a feather of a 24 days Khaki Campbell duckling. Fig. 17 shows the crescentic base, with whorls of dermal cells and the characteristic blood supply; fig. 18 is in the region

of the calamus; fig. 19 shows the fusion of the rhachis with the hyporhachis; fig. 20 shows the hyporhachis to be much shorter than the rhachis, as the former has almost disappeared from the section while the latter is still very large; and fig. 21 is near the tip of the feather where the aftershaft is represented only by the tips of its barbs, and the rhachis has branched repeatedly, but without any great reduction in size. The withdrawal of the pulp and cylinder cell layer can also be traced in this series. Figs. 22-26, Plate 18, represent sections taken from corresponding levels in a preplumule of the same age. It will be noticed that the structure is exactly the same as in the prepenna, except for the reduction in size and in the number of barbs. The minute feather germs of the duckling, which probably are prefiloplumes, also have the same structure as the prepennae, but even smaller in size than the preplumulae.

The feather is now structurally complete, except for the full length of the calamus but this is obtained by cornification of the intermediate cells below the short calamus found on the 24th day. Before hatching, when the feather sheath splits and the barbs splay out, the pulp is withdrawn to the base of the feather. This process is very gradual, commencing about the 15th day, when as fig. 8 shows, the pulp near the tip of the feather is relatively less dense, and the cylinder cells have begun to withdraw from between adjacent ridges. When the tip begins to cornify, the pulp (always bounded by cylinder cells) shrinks towards the centre, but generally remains attached by the cylinder cells, in one or two points, to the sheath between two barbs. It remains in this condition at the tip of the feather for several days, while the preliminary stages of withdrawal are taking place lower down, as cornification proceeds downwards. The crowded barbs and barbules push inwards to occupy the space vacated by the pulp, losing their regular arrangement. About three days before hatching, the pulp is entirely withdrawn from the tip, but is still present at the top of the calamus, where the first "cap" is formed. The formation of feather caps is discussed in the development of definitive feathers (p. 163).

28th day—On hatching the feather filaments dry, but friction, however slight, is necessary for the sheath to split. When once the dried sheath is slightly torn, the crowded barbs and barbules within push outwards, thus tearing away the sheath to the base and presenting the appearance seen in the protoptile of fig. 28, Plate 19.

(ii) Goslings

Only four stages of development of the goose were obtainable, aged 11, 14, 25, and 27 days respectively. The last was dead when opened on the 28th day, but the material taken from it did not appear to have suffered by the delayed fixation.

The development of protoptiles in a goose is essentially similar to that of a duck, differing only in very small points.

11 days—Although the 11 days embryo showed no signs of feather papillae in any part of the body (skin being examined from the spinal tract, tail, and wing), development must have been relatively more rapid than in the duck, where feathers

first show in sections of 8 days embryos. On hatching on the 28th day, the feather filaments of the gosling are fully formed and much larger and stronger than those of the duckling.

14 days—The embryo feather corresponds to a 12 days duckling or a 10 days chick feather. It is elevated above the general level of the skin, and with a steeper slope on one side. This is presumably before the backward growth has commenced, and correlated with it is the absence of a follicle. Near the tip of the feather, groups of intermediate cells are seen, merging lower down into a common band. The cylinder cell layer is indistinguishable in this region; apparently it does not appear until the intermediate cells have become arranged in groups. This supports the theory that it is the intermediate cells, which actually group themselves together, instead of being cut into ridges by the ingrowth of the cylinder cells.

Pigment cells containing very coarse granules, unlike the fine-grained pigment of the duck, are present near the apices of the ridges, and among the outermost intermediate cells near the base of the feather.

The stratum Malpighii is separated from the pulp by the bases of the cells forming a clear space (the "basal membrane") through the withdrawal of nuclei towards the periphery. A definite sheath, several rows in thickness, is present at this stage, and sections through the tip of the feather show this to join above the termination of the ridges.

The pulp is concentrated only at the base, and especially on the steeper side of the feather papilla. Near the tip of the feather it consists of very scattered nuclei connected by cytoplasmic threads, which indicates a comparatively earlier withdrawal of pulp than in the duck protoptile.

25 days—The feather follicle is well developed and the space between feather and follicle is occupied by a common sheath about six rows of cells in width. Cornification is in full swing in the upper part of the feather, the cylinder cells having withdrawn from between adjacent ridges, and now enclosing a small central pulp. Two large medullated and cornified barbs are present in the upper part of the feather filament, with progressively smaller barbs on either side. Lower down, these two large barbs fuse, forming the rhachis with which one or two of the neighbouring smaller barbs also fuse. In this way the feather consists near the base of a very broad rhachis, while the ventral part is still divided into smaller barbs. These rapidly fuse, resulting in the narrow hyporhachis or aftershaft. One side of this fuses with the rhachis, while a few barbs of the aftershaft are still free on the other side. This one-sided fusion seems characteristic of geese, although it occasionally occurs in the fowl. The lower region of the feather germ is still uncornified, and consequently the fusion of barbs can be clearly seen.

Each barb is circular in section, like the barbs of the duckling protoptile, and consists of elongated cells nearest the periphery, and more rounded cells towards the apex. During fusion the elongated cells join the circles of intermediate cells, which are not distinguishable except in their slightly greater width from the sheath cells. No barbules are present in this region. The calamus is succeeded by a broad

band of intermediate cells in the base of the feather, and unlike the condition on the 14th day, the cylinder cell layer is here quite distinct.

The musculature between the feather follicles is very definite, strands running from the top of one follicle to the base of the next. Traces seem to pass between both preplumulae and prepennae, but never so distinctly as between two prepennae.

28 days—The dead specimen of a Chinese gosling opened on the 28th day is covered with fully formed feathers, similar in structure to those described for the 25th day, but completely cornified (except for points of fusion), and with a longer calamus. The latter has the same crescentic base in transverse section as a duckling protoptile, but with the convexity facing the side, instead of towards the surface of the skin. This may be associated with the fact that the follicle of a goose protoptile is much deeper and correspondingly more oblique than that of a fowl. Pulp is still present, although withdrawn towards the centre in the tip of the feather.

The most striking feature of this gosling is the clearly defined blood system ; the course of the single central blood vessel surrounded by a circle of anastomosing capillaries is easily followed. In the definitive feather, the central vessel is clearly seen to be an arteriole, and the anastomosing vessels in the periphery are venules.

(iii) Chickens

6 days—Feather papillae appear on the 6th day of incubation in the chick, when sections of the skin of the spinal and femoral regions show similar concentrations of the dermis, and slight elongations of the Malpighian cells as on the 8th day in the duck and about the 12th day in the gosling. The developmental stages are similar to those described for the duckling, although always more precocious (correlated with earlier hatching).

10 days—The downgrowth of the epidermis which culminates in the feather follicle begins ; and the following day sees the formation of ridges. In the duckling and gosling these processes are reversed and the follicle is formed about two days after the ridges.

11 days—In sections of an embryo on this day of incubation, pigment is found scattered about the epidermis surrounding the follicle, the walls of the follicle and the intermediate cells of the feather germ. Thus pigment must be of endogenous origin, as it is found in the epithelium with no previous traces in the dermis.

12-16 days—The regrouping of intermediate cells to form barbules and barbs occurs on the 12th day, and by the 16th day the barbs are well developed and medullated, while the barbules are very crowded. The shape of the barbs is characteristic, being oval in cross-section, fig. 11, Plate 17, unlike the rounded barbs of duck and goose, fig. 9, Plate 17. But, as in the duck in early stages of development of the barbs, a point may be seen projecting from the side opposite the apex of the ridge, which is attached to cytoplasmic strands, as though intermediate cells have lately been drawn into the barb. There are very few medullary cells in these barbs, the central spaces rarely being divided.

The chief difference in the development of protoptiles in ducks and fowls lies in the fact that the former have a well developed rhachis and hyporhachis, while the latter have a very short rhachis, and therefore fusion of barbs does not occur until late stages of development (about the 19th day). Consequently, for a considerable distance from the tip of the feather, the uniform barbs (of which only about three in shaft and aftershaft are slightly larger than the rest) are crowded indiscriminately into the space vacated by the pulp just before hatching. The calamus is very short and no caps are formed during the incubation period.

21 days—Some time after hatching, the protoptiles are freed from their dried sheaths, and the barbs spread out, forming a dense covering of soft, warm down.

(c) *The Development of Teleoptiles in the Wings of the Chick*

Feathers first appear microscopically on the wings of the chick about the 10th day of incubation, but their development is relatively more rapid than the development of the protoptiles on the rest of the body. About the 13th day, a second generation of feathers makes its appearance below the prepennac of the remiges. These are the teleoptiles, which on hatching are equal in length to their predecessors which they still bear on their tips, fig. 27, Plate 19.

The first signs of teleoptiles are seen in longitudinal sections of the wing when the follicle of the protoptile becomes greatly elongated, extending to the position of the differentiating cartilage. The sheath, the intermediate cell layer below the short calamus of the protoptile and the cylinder cell layer are also continued downwards, retaining their connexion with the sheath and Malpighian layer of the follicle. The dermis becomes greatly concentrated in this region, and meets the dense pulp at the base of the protoptile.

The calamus of the protoptile is continued for a short distance further on the upper side of the feather, resulting in a crescentic shape, when seen in transverse section. On the opposite side the intermediate cells become aggregated together to form the ridges of the teleoptile, inside the narrow inferior umbilicus of the protoptile. Thus transverse sections at a later stage show a few barbs of the teleoptile in the same section, but on the opposite side of the feather germ to the barbs of the protoptile, fig. 16, Plate 17. Formation of ridges continues, and certain of these on the dorsal side fuse at intervals, giving rise to the rhachis. As may be seen in fig. 12, Plate 17, barbs from each side of the rhachis fuse with it almost simultaneously.

Pigmentation and differentiation of barbules and barbs take place as in the protoptiles, but the resultant shape of the barbs is much more elongated than in the protoptile. This is probably due to the presence of a greater number of barbs inside a sheath of only slightly greater diameter than that of the protoptiles. Correlated with this is the relatively thin cortex of the barbs, only the apex and opposite end retaining their characteristic thickness. The proximal barbule cells are comparatively long in transverse section, fig. 13, Plate 17, resulting in the broad flattened base of the fully formed barbules seen in fig. 29b, Plate 20. The hamuli,

which make their first appearance in the teleoptiles are possibly due to the crowding of barbules in very early developmental stages, resulting in the curvature of the more distal cells.

Towards the tip of the teleoptile, the ridges decrease slightly in size on either side of the rachis. No ridges are present on the ventral side of the feather germ, but they appear lower down, fusing near the base to form the short aftershaft. Fig. 10, Plate 17 shows a transverse section of this region, the smaller barbs of the left hand side representing the aftershaft; fig. 14, Plate 17, shows the tips of the barbs of the aftershaft; fig. 15, Plate 17, is a section showing the main shaft persisting to the tip of the feather, and fig. 16, Plate 17, illustrates the manner in which the barbs of the teleoptile are present at the same time as barbs of the protoptile.

It has been suggested that there is direct continuity between a few barbs of the teleoptile and the calamus of the protoptile. Although this is certainly true of the duckling, in the chick, however, by following serial transverse sections from base to tip of the feather it is seen that the barbs of the former have disappeared, though the distal ends of the barbules are still present when the barbs of the protoptile appear. It seems equally probable that the manner in which protoptiles are carried for some days on the tips of the teleoptiles after the latter have broken from their sheaths, is due to the narrow base of the protoptile constricting the developing barbs of the teleoptile. At any rate, the barbs of the teleoptile show a distinct curve distally when they first spring from the calamus of their predecessor, fig. 27, Plate 19.

Cornification takes place as in the protoptile but it has not reached the base of the teleoptile papilla when the chick is hatched.

The development of protoptiles is therefore fundamentally similar in the duck, goose, and fowl, minor differences being due to relative lengths of incubation periods, resulting in differences in times of appearance of the constituent parts of a feather, and to the differences existing between the specific forms of the protoptile on hatching. Bearing in mind, that the feather is continuously growing from the base, the stages in development may thus be summarized as follows:—

- (1) The concentration of dermal nuclei and the elongation of epidermal cells above this concentration.
- (2) Proliferation of the stratum Malpighii, resulting in the formation of intermediate and cylinder cell layers.
- (3) The backward elongation of the papilla thus formed and the formation of the sheath by the division of epitrichial and intermediate cells.
- (4) The aggregation of intermediate cells forming ridges, followed by the binding cylinder cell layer; the appearance of pigment cells, formed in situ in the epithelium; the formation of the feather follicle by the downgrowth of the epidermis.
- (5) The division of intermediate cells forming one median and two lateral plates in each ridge, the former giving rise to the barb and the latter to the barbules.

- (6) The differentiation of barb cells into medulla and cortex.
- (7) The fusion of barbs dorsally, forming the rhachis, and later the fusion of barbs ventrally, forming the hyporhachis.
- (8) The lateral fusion of the rhachis and hyporhachis, forming the calamus.
- (9) The withdrawal of the pulp and cylinder cell layer, and the cornification of the feather from the tip downwards.
- (10) The formation of the feather caps, and the elongation of the calamus by cornification of undifferentiated intermediate cells at the base of the papilla.

The teleoptile in the wing of the newly hatched chick develops from the same papilla as its proptile predecessor, and remains attached to it by continuity between a few barbs of the former and the calamus of the latter.

V—DEFINITIVE FEATHERS

The histology of definitive feathers is fundamentally similar in feathers from the different pterylae (*i.e.*, head, neck, breast, thigh, tail, wing, and back), and therefore will not be considered separately.

As might be expected from the study of nestling feathers, the epidermal layers giving rise to the definitive feather are all derivatives of the stratum Malpighii. The stratum corneum forms the sheath of the feather and its follicle; the stratum intermedium forms the appendicular parts of the feather, and the stratum cylindricum forms the feather caps. The dermal component, *i.e.*, the pulp, is, of course, merely a transitory structure.

The development of the definitive feather will therefore be considered under the following headings: (*a*) stratum corneum; (*b*) stratum intermedium; (*c*) stratum cylindricum; and (*d*) pulp, in both the domestic fowl and the starling.

(*a*) *The Development of Definitive Feathers*

(i) Fowls

(*a*) *Stratum corneum*

This uppermost layer of the epidermis may be distinguished in transverse section passing down from the general surface of the skin into the follicle, and at the base, of this, its continuity with the sheath of the feather is obvious. Contrary to the statement made by POULTON (1894) the feather sheath consists of not one, but sometimes as many as ten or more layers in thickness (*e.g.*, tail feathers). Cornification takes place simultaneously in the sheaths of both feather and follicle, and passes gradually down towards the base. It is practically impossible to distinguish one from the other, until this process is almost complete. Then, however, the slight amount of basal growth, which still takes place, causes the feather sheath to be pulled outwards and away from the follicle sheath. The close connexion between the sheaths of the feather and its follicle may be seen by the irregularity of the break

between them, layers adhering at one point to the follicle sheath, and the same layers adhering to the feather sheath at other points, fig. 30, Plate 21.

(b) *Stratum intermedium*

From this layer, the rhachis, hyporhachis, barbs and barbules arise, *i.e.*, all except the basal part of the developing feather (chiefly within the follicle) are formed from the stratum intermedium. The method of formation of these structures is fundamentally similar to that described for neossoptiles. Slight modifications, however, are present and are correlated with the different types of feathers produced (*i.e.*, from the thigh, back, wing, etc.), as distinct from their uniform downy predecessors. The structures arising from this layer will therefore be considered under separate headings: (1) ridges; (2) rhachis and hyporhachis; (3) barbs; and (4) barbules.

(1) *Ridges*—The formation of ridges in a definitive feather proceeds as in the nestling down, although (and especially towards the end of development) the barbule plates may be differentiated before the ridges are cut off. In rapidly cornifying feathers (*e.g.*, ear bristles) this acceleration of formation of parts may be carried even further, and the barbs and barbules cornified without any definite ridge formation, *i.e.*, with the cylinder cells completely encircling the pulp.

(2) *Rhachis and Hyporhachis*—As in the development of protoptiles, the rhachis and hyporhachis of the definitive feather are formed by fusion of barbs, fig. 32, Plate 21. In bilaterally symmetrical feathers, the so-called ventral point at which the hyporhachis arises, lies diametrically opposite the dorsal point. This is well seen in back feathers, fig. 30, Plate 21, in which the aftershaft is well developed. Almost halfway between the dorsal and ventral points (*i.e.*, halfway between rhachis and hyporhachis in transverse section) lies the region of plasmatic growth, fig. 31, Plate 21, where barbs destined to form either shaft or aftershaft arise. What causes their growth in a dorsal direction towards the rhachis, or in the opposite direction towards the hyporhachis is uncertain, but it appears that the intermediate cells are divided primarily into four large ridges—one on either side of the dorsal point, and one on either side of the ventral point, fig. 30, Plate 21. These are then secondarily divided into the small ridges giving rise to the barbs and barbules and all the barbs in the large ridge on either side of the dorsal point fuse to form the rhachis, while all those in the large ridge on either side of the ventral point ultimately fuse to form the hyporhachis. In feathers with rudimentary aftershafts, this is less obvious but still apparent.

The remiges of the fowl are an instance of asymmetry due to the deflexion of the ventral point from its position diametrically opposite the dorsal point, fig. 53, Plate 28. This results in the vane being narrower on one side than on the other, and in a slight curvature of the rhachis. In remiges and retrices, the rhachis may be so disproportionately large as to occupy the greater part of the pulp cavity.

The actual structure of the rhachis varies in different types of feathers. In filoplumes and in down feathers it is solid, very rarely in the latter but never in the former, having traces of medullation.

In pennaceous feathers of all descriptions, the rhachis is always medullated. The outer cortex, which in coloured feathers invariably receives the greatest amount of pigment, is uniformly cornified early in development, as in nestling down; while in the region nearest the pulp, as might be expected, cornification is retarded, and cell structure is still visible when barbs, barbules and the dorsal side of the rhachis are completely cornified.

In large feathers (*e.g.*, remiges and rectrices) and in the basal region of smaller ones, projections from the cortex extend into the medulla of the rhachis, presumably forming stiffening rods. These have no connexion in number or time of origin with the fusion of barbs.

The medulla consists, as in nestling down, of large vacuolated cells, fig. 32, Plate 21. In very rare cases, small deposits of pigment may be found within these cells, usually restricted to the region where the cytoplasm containing the nucleus withdrew at the commencement of vacuolation.

(3) *Barbs*—Barbs differ greatly, in transverse section, in size and shape in different types of feathers and according to their position. In down feathers or the downy region of contour feathers, they are rarely if ever medullated, and typically consist of barrel-shaped masses, becoming slightly elongated when nearing the ventral point.

In pennaceous feathers, the barbs are invariably medullated for the greater part of their length. The medulla, absent at the extreme tip, usually disappears again before fusion occurs with the rhachis. Continuity has not yet been observed between the medulla of barbs and rhachis.

Near the ventral point, barbs tend to be round or barrel-shaped, becoming progressively elongated in transverse section, in nearing the rhachis, fig. 30, Plate 21. In the rounded region, the medulla may be several cells wide; yet towards the base of the barb, always consisting of a single layer of cells, fig. 32, Plate 21. As in the rhachis, pigment is invariably more densely deposited on the outer side of the barbs.

It is almost certain that cells which are destined to form the barb are the pigment cells in early stages of development. It is invariably in that region of the ridge which ultimately gives rise to the barb, that the pigment cells are found, fig. 31, Plate 21, sending pigment granules by means of long pseudopodia to the barbule plates. The latter are formed before the barb plates, which usually arise after pigmentation is complete. Then, possibly through loss of pigment to the barbules, the pigment cells are much smaller and less dense than before, and these cells are incorporated into the barb plate, thus losing their identity. This possibility of pigment forming in situ in the cells of the barb itself, has been previously mentioned by LILLIE and JUHN (1932).

The origin and fate of pigment cells which have been observed among the cylinder cells surrounding the rhachis, fig. 32, Plate 21, just before cornification of the latter, is uncertain.

(4) *Barbules*—These too differ in size and shape according to position and type of feather. In down feathers and the downy basal areas of contour feathers, they

are usually square in cross-section, and appear to be attached end to end. On cornification, this attachment is broken, and each barbule square becomes a separate unit.

In the lacey tips of feathers, barbules appear as narrow elongated cells in cross-section, but in the compact region of the vane, the hooked ends of the barbule cells form the hamuli. This differentiation of barbules has been claimed as a possible means of distinguishing the sex of the fowl by studying the germs of such feathers as the neck hackles, which have a broader lacey tip in the cock than in the hen.

The hooked barbules arise at a lower level on the side away from the rachis than the straight barbules of the opposite side, fig. 33a, Plate 21. This is to be expected from the interlocking method of barbules, where the upper barbules of one barb hook on by means of their hamuli to the slight groove on the lower lying barbules of the adjacent barb.

Pigmentation of barbules takes place relatively early in development, and dense masses of granules may be seen encircling the nucleus. As the barbules elongate, the pigment invariably becomes aggregated at the end which is distal with reference to its attachment to the barb.

(c) *Stratum cylindricum*

During the early development of a feather, this layer proliferates and adds a few cells to the rapidly multiplying intermediate cells. But its later function is quite passive, as it merely acts as a binding layer, which follows the divisions of the intermediate cells into ridges, or in the region of the calamus, retains its circular nature.

Immediately following the onset of cornification, the cylinder cells play a more conspicuous though still passive part. Cornification and withdrawal of pulp proceed simultaneously whatever the type of feather, and they are definitely correlated. The conditions initiating keratinization are still obscure, though attempts have been made to explain it in hairs by means of a cystine gradient (KING and NICHOLLS, 1932).

The cylinder cells at this time are still in an obviously healthy condition, and retain their normal cytoplasm and nuclei, although they have long since ceased to divide and may be stretched out of their typical shape. As the pulp withdraws, the cylinder cells remain attached to the still living material, and are pulled away from the cornified barbs and barbules. That a definite pull is exerted by the pulp is seen in the stretched and broken cytoplasmic strands connecting the cylinder cells with the intermediate cells in the early stages of withdrawal.

As the pulp passes down the feather, the cylinder cells become congested, and the outermost ones, being further from the nourishment contained in the abundant blood supply of the pulp, are overtaken by the process of cornification. This occurs at the sides as well as the tip of the pulp, fig. 37, Plate 22, so that a series of cones is formed, connected with each other.

In this way the feather caps arise, and, where protected by the calamus, persist, although distal to the superior umbilicus they soon break away from the feather.

DAVIES (1889) describes the method of cap formation from an entirely different point of view. He considers the cylinder cells to recommence dividing at certain stages during withdrawal, when the pulp is stationary. He does not explain what causes the pulp to remain at a certain level, and then suddenly to continue passing proximally after such a pause, nor why cells which have remained passive for so long should suddenly start dividing again when comparatively far from nourishment.

(d) Pulp

In a developing feather, the dermis is concentrated round the follicle, and also, and to a greater extent, within and below the base of the feather germ. In successive cross-sections through the base of the feather, this aggregation of whorls of dermal cells may be seen to extend for some distance below the actual base of the feather. This basal region is the growing part.

Nourishment is carried to the developing feather by means of a definite blood supply in the pulp. In a large definitive feather, *e.g.*, a remex or rectrix, the blood system differs from that of an embryonic down feather only in its greater complexity. The general plan of a central arteriole branching at the top of the pulp into numerous anastomosing venules still persists, but the arteriole of a definitive feather may be duplicated and occasionally branch, while the venules are more extensive.

This dermal component of a feather is transitory and as soon as its function of nourishing the developing barbs and barbules is finished, its withdrawal commences. As already stated, this occurs at the same time as cornification begins in the peripheral regions of the tip of the feather, starting at the sheath and passing inwards.

The circulatory system is entirely remodelled during the withdrawal of the pulp, tiny vessels fusing to form large blood spaces which often occupy the whole of the space beneath the developing feather cap, and may be left there when the pulp withdraws further.

This might be thought by adherents of DAVIES's theory of cap formation to be an incentive for cylinder cell division ; but these cells may form many congested layers, and cornification, having once started, seems to gain speed in passing downwards. Hence the accumulation of blood sometimes occurring at the top of the withdrawing pulp can have little effect on the outermost cylinder cells.

The withdrawn pulp is absorbed by the dermis, and no trace of its previous extension within the developing feather exists, except for a small papilla projecting within the inferior umbilicus. Immediately before the moulting of a feather, however, the quiescent pulp recommences growth and keeps pace with the developing intermediate cells up to the mouth of the follicle.

The pulp has been considered (LILLIE and JUHN, 1932) to be constantly growing at its base, and constantly dying off and being resorbed at its apex. From the fact that both in longitudinal and transverse sections the pulp may be seen to be actually torn away, first from the barbs as they move upwards, and secondly from the feather caps in process of formation, fig. 37, Plate 22, it would seem more probable that the pulp actually withdraws and is resorbed at the base, as earlier authors (DAVIES,

1889 and STRONG, 1902*a*) have observed. This is confirmed by the fact that growth of the pulp is no longer necessary at this time, as when pulp withdrawal takes place, the whole of the feather (except the calamus) has been formed, and only cornification is to be completed.

(ii) Ducks

The development of definitive feathers in the Khaki Campbell duckling was studied for the first eight weeks after hatching. In general, this process is similar to that of the fowl, the chief differences being in the relative times of appearance of the second generation of feathers. In the duckling, the wing feathers are retarded in development and do not appear externally until the 5th week. The tail teleoptiles however develop during the second week at the base of the greatly elongated calamus of the protoptile. These are the only feathers which show major structural differences from the fowl feathers already described.

Sections through the tip of a tail protoptile show the rhachis to be indistinguishable from neighbouring barbs, but more proximally, a one-sided fusion takes place rapidly followed by a second fusion on the opposite side. The median barb may therefore be regarded as the rhachis, and after this preliminary fusion, it is always characterized by its greater size. At the proximal end of the protoptile, the rhachis may occupy one half of the transverse section and form one side of the circular calamus. It then becomes much narrower, leaving the calamus of uniform width. This marks the distal limit of the elongated calamus peculiar to the rectrices.

The rectrices of the duckling are of particular interest in that there is no definite line of demarcation between protoptile and teleoptile. The barbs of the former fuse to form rhachis and hyporhachis, which in turn fuse to form the calamus. The calamus then splits up into barbs, fig. 28, Plate 19, which fuse to form the rhachis and hyporhachis of the teleoptile. A slight constriction is usually present at the base of the calamus of the protoptile, probably marking the site of the inferior umbilicus, and below this the calamus splits into the barbs of the succeeding definitive feather.

It is possible that this peculiar state of affairs is due to the rapid growth of the teleoptile. Usually a quiescent period sets in between the formation of the two generations of feathers, but the feather papilla of the rectrix of the duckling is obviously in a very active state throughout the nestling period.

This offers striking proof of the facts disputed by previous authors that (*a*) the second generation of feathers is formed in the same follicles as their predecessors, and (*b*) that the feather papilla resumes activity prior to the shedding of the old feather.

(iii) Starlings

The first coat worn by the starling consists of a very rudimentary nestling down similar fundamentally to that of the chick. It was unfortunately impossible to obtain sufficiently early stages to study the development of these protoptiles.

The youngest stage obtained had feathers visible macroscopically on the head and round the eyes and ears ; on the neck, ventrally two rows of feathers converging below the head and extending backwards almost to the cloaca, and dorsally, extending from the head to the tail in the middle line ; on the thighs and wings, with a few on the legs.

None of these feathers had emerged from its follicle, but could be seen by means of the pigment contained, or by the raised lumps which they formed on the surface. All follicles, however, had downy protoptiles protruding.

The largest specimen obtained had primaries measuring 4 cm, with other feathers proportionately long, and all having burst from their sheaths for the distal quarter of their length.

(a) *Stratum corneum*

This layer forms a thick sheath to the feather and a thinner sheath to the follicle, but they are much more widely separated than in the fowl.

(b) *Stratum intermedium*

Residual cells are more definite and in larger numbers than in the fowl. In longitudinal sections of primaries the barbule plates of each ridge are seen bounded by cylinder cells and with a central layer of residual cells lying between them, in appearance very similar to the true cylinder cells. These join up a triangular colony of cells near the sheath to the developing barb. Presumably the residual cells are either cornified with the sheath, or atrophy in the centre of the ridge.

As in the fowl, the shape of barbs and barbules differs according to the position with regard to the rhachis, its level in the feather and the position of the feather on the body.

In wing and tail feathers, the barbule plates nearest the rhachis consist of very long, deeply pigmented cells, with blunt inner edges and narrow outer ones. On the far side of the barb from the rhachis, the barbules are narrower, less pigmented and hooked, and develop later than do their neighbours.

In thigh feathers, the barbules are wedge-shaped and do not bear hooks.

(c) *Stratum cylindricum*

This layer typically consists of cells with rounded nuclei containing two to three nucleoli. Between the ridges, the rounded shape may be lost and the cylinder cells represented only by a double narrow, nucleated string. This is undoubtedly due to the close proximity of the ridges.

(d) *Pulp*

The formation of feather caps and the withdrawal of the pulp take place as in the fowl. Fig. 37, Plate 22, shows the pulp actually pulling away from the developing cap, in a tail feather of a starling.

Thus development of definitive and nestling feathers differs only in that the latter arise from the general epidermis of the body, while the former already have the

dermal papilla sunk within a follicle. Differentiation of the epidermal covering of this papilla takes place in the same way as differentiation of analogous structures in the embryo, with modifications resulting in the different types of feathers found in the adult.

(b) *Moulting and Replacement of Feathers*

The development of regenerating feathers does not appear to have received much attention. As LILLIE and JUHN (1932) have stated regarding the papilla, "it has not been known whether its activity is resumed prior to actual moulting . . . or whether the shedding of the feather is the actual stimulus to renewed activity".

The usual method employed in the study of plumage variations is to pluck the feathers from a given area before injecting or feeding an endocrine substance, and then to compare the regenerated feathers with normal ones.

For these reasons it has been thought advisable to study the feather germ (a) under normal conditions (*i.e.*, after moulting) and (b) after deliberate plucking (Part III). In this section, normal regeneration is considered.

Sections were taken of young feathers developing in the follicles of feathers which were about to be moulted, or which had recently been shed. Such stages are easily obtained in the fowl up to the assumption of the adult plumage, as no definite moult occurs between the different coats worn by the young animal, since a gradual shedding of old feathers and replacement by others of a slightly different type takes place.

On emerging from the follicle, the new feather is still covered by a sheath, which is crowned distally by a small hook-like projection. Serial transverse sections through this region show the hook-like projections to consist entirely of sheath and a layer of cornified cells similar to a feather cap, and therefore presumably consisting of cylinder cells. Lower down, the tips of the barbs and barbules appear on one side only (*i.e.*, the dorsal side) between approximately the 3rd and 4th layers of cornified cells from the centre. More proximally, pulp is seen beneath a definite feather cap, and below this region, the feather is formed in the usual way.

A longitudinal section through such a feather may show as many as five caps between the tip of the feather and the pulp, and the narrow cornified layers going up to the cap are continuous with the stratum cylindricum and the stratum intermedium. The actual tip is sheath only, and therefore represents the stratum corneum, fig. 34, Plate 22.

Figs. 35 and 36, Plate 22, show the method of development of this tip. The calamus of the old feather becomes much narrower and slightly constricted in nearing the base, and into this constricted region (the inferior umbilicus) the papilla projects. The epidermis covering this is complete in all its layers. The cylinder cells are continuous with the cylinder cells of the follicle walls and are quite separate from those (now cornified) of the last feather cap. The intermediate cells are similarly continuous, and already show signs of ridge formation. Finally, the stratum corneum

of the papilla is continuous with the sheath cells of the follicle and with the extreme base of the old feather.

The manner in which the complete epidermal covering of the tip arises may be followed from serial transverse sections. Near the base of the feather the calamus becomes narrower, owing to cornification of the outer intermediate cells only. The inner layers become slightly vacuolated, so that a new sheath is formed within the calamus. This is, naturally, continuous at the base with the sheaths of the old feather and its follicle, although the former is very narrow and scarcely distinguishable from the calamus.

The order of layers from the follicle sheath inwards now consists of feather sheath, calamus, sheath of new feather, intermediate cells, cylinder cells and pulp. As the pulp withdraws and the last feather cap is formed, the intermediate cells within the calamus are drawn over the tip of the pulp together with the cylinder cells above which they lie. This then forms the new papilla, the old calamus breaking away from the new sheath cells laterally.

It is evident, therefore, that the feather papilla resumes activity prior to the shedding of the old feather, and this is most conspicuously demonstrated by the protoptiles borne on the tips of the teleoptiles in the fledgling.

VI—DISCUSSION

These investigations deal with three main groups of problems associated with (a) sequence of plumage, (b) histological development of feathers, and (c) the actual structure of a feather.

(a) Sequence of Plumage

The sequence of plumage in birds has been the subject of discussion at various times. In 1907, LYND'S JONES pointed out that "the first down and its succeeding definitive feather are produced by one continuous growth, and therefore cannot be regarded as two distinct feathers. The first down is the plumulaceous tip of the definitive feather."

At a cursory glance this might appear true, as invariably the protoptile is borne on the tip of its successor for some time; but, except in areas where the development of the second coat is accelerated (as in the remiges of the fowl), the fact that a definite calamus exists at the base of the protoptile, into which the distal barbs of the second generation feather project as in the fowl, or with which they fuse as in the duck, shows the protoptile and its successor to be distinct feathers produced consecutively by the same papilla.

The sequence of plumage in the Brown Leghorn fowl has recently been studied by DOMM, GUSTAVSON and JUHN (1932) who describe four stages, down, chick, juvenile, and adult; but "because their development is a gradual piece-meal affair, succeeding stages are invariably intermingled before the attainment of the adult plumage".

The chick plumage differs from the down, according to these authors, by the precocious development of remiges and rectrices, together with a few feathers along sides of breast and belly. Now in the Rhode Island Red and the Black Leghorn X Light Sussex, and according to FINN (1919) in all Galli, the remiges are developed on hatching, and breast and abdominal feathers appear later. Thus the "down" stage may be considered as relegated to the incubation period. In some breeds, e.g., Rhode Island Red, the rectrices do not usually appear until after the fourth week in the male birds, although present in the female during the second week. The difficulty of dividing the plumage into definite stages is thus obvious.

CLARKE as early as 1906 announced the discovery of two coats of nestling feathers in Ringed and Gentoo penguins, and in 1907 PYCRAFT assumed that most common birds have lost their protoptile coat, while in Ratitae the mesoptiles may persist in the adult. INGRAM (1920) suggests that it is the second coat which is lost in most birds, and COSSAR EWART's work on the Mallard duckling (1921) corroborates this.

As already noticed, the neossoptile coat exists in members of Anseriformes, Galliformes, and Passeriformes, but while EWART (1921) has figured three definite coats in the first of these groups, it has so far been impossible to find them all in the others.

The mesoptile is usually portrayed (e.g., EWART's duck) as a structure intermediate in type between the down feather and the pennaceous form. In the chick it was at first thought that the wing feathers present on hatching represented mesoptiles, but their later development showed them to be similar to the adult type of feather in structure. Differences of course exist, but since these consist of shape of vane or pigmentation—which vary considerably in the adult—they can hardly be called characters defining generations of feathers.

The question then arises as to whether the nestling down of the chick consists of protoptiles or mesoptiles. NEWTON (1896) defines a typical protoptile as consisting of :—

- (a) a very short calamus ;
- (b) an insignificant or ill-defined rhachis, if there be one at all ;
- (c) the almost universal absence of cilia, and
- (d) the absence of an aftershaft, except in *Dromaeus*.

Now according to EWART (1921) the nestling down of the Mallard duck consists of feathers characterized by :—

- (a) a well-developed calamus which may have as many as 20 cones,
- (b) a well-developed rhachis,
- (c) well-developed, sometimes hook-like cilia,
- (d) short, stiff as well as long, slender barbs, and
- (e) a well-developed aftershaft, the barbules of which bear cilia.

From the present investigation, the nestling down of the Khaki Campbell and Aylesbury ducklings and the Chinese gosling is in these respects similar to the Mallard duckling, and hence probably to all Anseriformes.

The chief difference between the nestling down of the chick and the duckling lies in the ring-like swellings at the nodes of the barbules in the former, and the presence of cilia (*i.e.*, projections from the nodes) in the latter.

It may thus be concluded that EWART's description of a typical protoptile is more accurate than the earlier one of NEWTON.

On this similarity of all essentials in the first coat of the chick and the duckling, we may assume the nestling down of the chick to consist of protoptiles, and therefore the mesoptiles have been suppressed.

In the Mallard duckling and the Chinese gosling, EWART claims to have found all three types of feather in continuity with each other. The proximal and youngest feather represents the teleoptile; the median feather, which is definitely less highly specialized than its successor, being the mesoptile, and bearing on its tip the first nestling down or protoptile. No such intermediate form has been found in the chick.

Not only is the mesoptile coat totally suppressed in the chick, but in the region of quickest growth of feathers (*i.e.*, primaries and secondaries) the protoptile coat is less highly developed than elsewhere. This refers only to the completion of the calamus, which usually grows to contain several caps, but the precocious formation of the teleoptiles of the remiges prevents more than the beginning of the calamus from being formed.

(b) *The Histological Development of Feathers*

The attention of earlier writers in this field was mainly concentrated on such points as ridge formation and pigmentation, while modern work stresses the importance of the method of formation of the rhachis. Hence these three problems will be considered separately.

(i) Ridge Formation

The problem of ridge formation has resolved itself into the question whether the grouping of intermediate cells to form ridges is due to an invasion of the intermediate cells by the cylinder cells, or whether the intermediate cells themselves first form groups, the cylinder cells merely retaining their limiting position. Both DAVIES (1889) and STRONG (1902 *a*) consider the last condition most probable, as the intermediate cells may be seen to change their position during the early stages of barbule formation. Again MAURER (1892) and STRONG (1902 *a*) agree that the rapid growth of intermediate cells causing increased pressure on the pulp, may be a factor in ridge formation. In later stages of development of protoptiles, ridges may be formed before the cylinder cell layer is differentiated from the intermediate cells; and cylinder cells are not present between ridges when they are fusing to form the rhachis.

In the development of teleoptiles, barbs and barbules may be formed from the intermediate cells before any ridges exist, and in some feathers, cornification proceeds so rapidly that only attempts at ridge formation are possible. In thigh and back feathers with very large aftershafts, the ridges are crowded into such a confined space that there is little room for cylinder cells to extend from the apex

of the ridge to the sheath, figs. 30 and 52, Plates 21 and 28. They may be traced for a certain length and then become indefinite, and it is doubtful whether the ridges are ever quite separated from each other near the periphery.

It is evident, therefore, that the intermediate cells take the initiative in forming ridges, and the cylinder cells later pass between them.

(ii) Pigmentation

The origin of pigment has been the subject of various theories but its distribution in the duck, goose, and fowl supports the view advocated by DEMIÉVILLE (1884), MERTSCHING (1889), JARISCH (1891 and 1892), RABL (1894), POST (1894), ROSENSTADT (1897), LOEB (1898), PROWAZEK (1900), and STRONG (1902 *a*). These authors believe it to be the result of metabolic activity of either the nucleus or cytoplasm of epithelial cells.

STRONG, working on the definitive feathers of *Sterna hirundo*, found pigment first appearing in the intermediate cells before the formation of ridges, and no pigment in the pulp. These results were based on sections cut at different levels of a fully formed feather, and consequently the absence of pigment in the pulp might be due to its migration outwards between the cylinder cells and so to the intermediate cells, as writers in favour of the exogenous (*i.e.*, dermal) origin of pigment believe. However, in the sections of embryonic and definitive feathers studied in the present enquiry pigment first appears in the intermediate cells after the formation of ridges, and no traces are found in the pulp. Further, in the goose and fowl, pigment may be present in the epidermis surrounding the follicle, but not in the dermis. In hairs, pigment has also been found appearing first in the Malpighian layer of the papilla (SPENCER and SWEET, 1899).

Pigment appears concentrated towards the outermost intermediate cells nearest the sheath, in the earliest stages at which it is present in embryonic feathers, and in successive days of incubation, wanders towards the apex of each ridge, from there sending out amoeboid processes along which pigment granules flow, fig. 5, Plate 16. The word "flow" is used since different concentrations of pigment are visible in different parts of the processes. This tendency of pigment cells to move inwards from their place of origin, accounts for isolated pigment cells which have been found on extremely rare occasions in the pulp of duckling and gosling feathers.

The fact that in definitive feathers pigment cells may be incorporated into the barb as normal cells, also supports the view that in feathers pigment is formed within the epithelial cells.

(iii) Rhachis

The study of the development of both nestling and definitive feathers confirms the theory advanced by DAVIES (1889) and STRONG (1902 *a*) that the rhachis is formed by fusion of barbs. This method of formation of a feather is illustrated in fig. 56. The first ridges formed give rise to barbs of approximately equal size, and at this point (*i.e.*, the future tip of the feather) it is impossible to distinguish the primordium of the rhachis, fig. 56A. This condition extends for a very short distance,

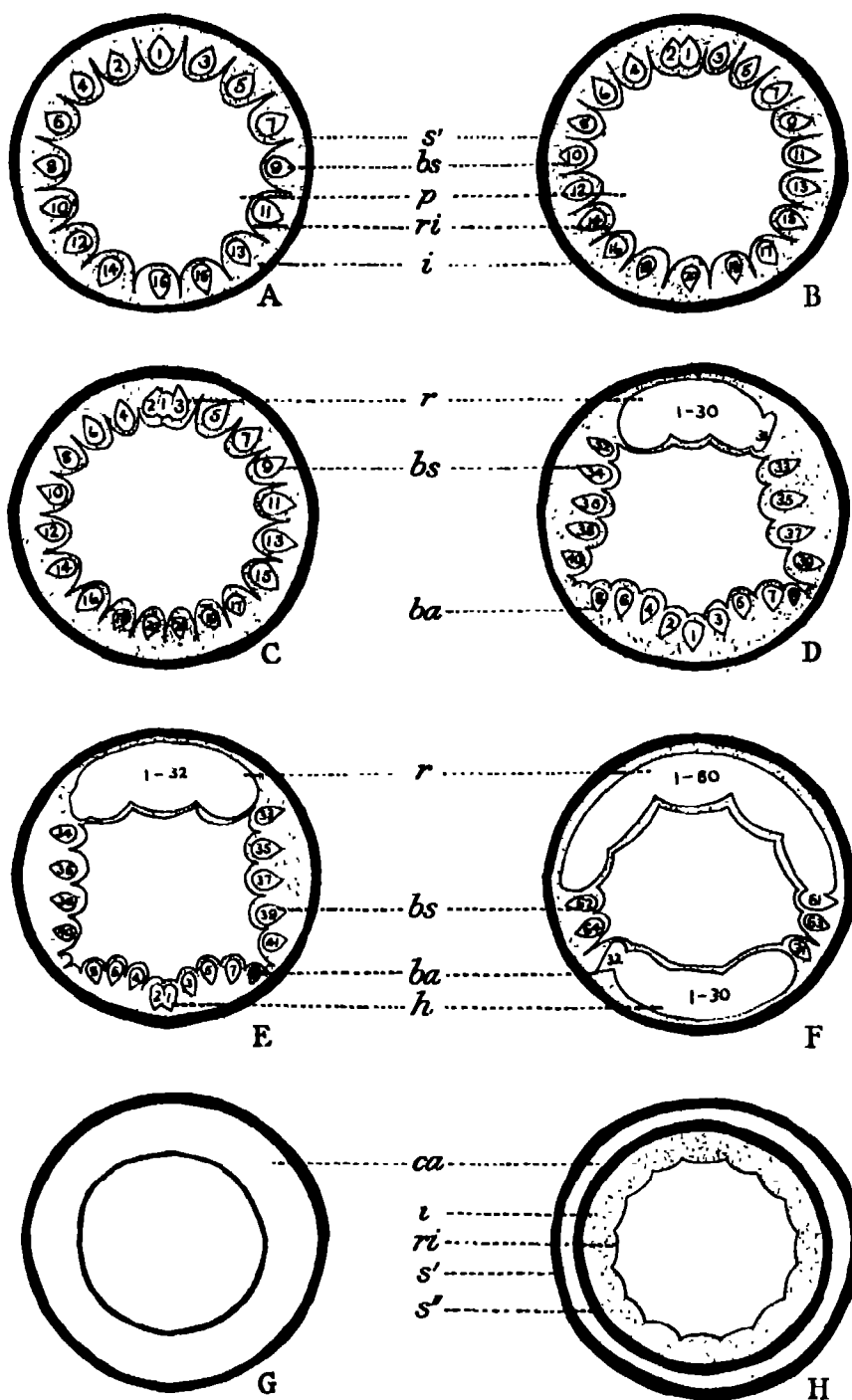


FIG. 56—Diagrams of cross-sections of a feather to show the dorsal fusion of barbs to form the rachis (A-F); the fusion of barbs ventrally to form the hyporhachis (D-F); the lateral fusion of rachis and hyporhachis to form the calamus (F); the structure of the calamus (G) and the tip of the new feather forming within the base of the calamus of its predecessor (H). *ba*, barb of aftershaft; *bs*, barb of shaft; *ca*, calamus; *h*, hyporhachis; *i*, intermediate cells (i.e., collar); *p*, pulp; *ri*, ridge; *s'*, sheath of old feather; *s''*, sheath of new feather.

the length varying in different types of feathers, and the fusion of two of these identical barbs marks the beginning of the rhachis, fig. 56B. By repeated fusion, the rhachis becomes conspicuously large and may occupy the dorsal half of the feather papilla. Near the base (the distance again varying according to the type of feather) barbs arise on the ventral surface, which, like the first formed barbs on the dorsal side, show little difference in size, fig. 56D. Fusion of these barbs gives rise to the hyporhachis, fig. 56 E. The superior umbilicus is formed by the lateral fusion of the rhachis and hyporhachis, fig. 56 F, and proximally the fused structures form the calamus, fig. 56 G. Fig. 56 H shows the tip of the succeeding feather within the calamus of its predecessor.

(c) *The Actual Structure of a Feather*

Until quite recently, ornithologists have been uncertain as to the true composition of nestling down, and the generally accepted view was that no aftershaft is present (HEADLEY, 1895). EWART (1921) summarizes the reasons for regarding the aftershaft as an accessory and secondarily acquired structure, as follows:—

- (a) That the aftershaft is developed from a forward elongation of the calamus (according to GADOW) ;
- (b) that the tip of the aftershaft is never attached to the calamus of the feather about to be shed.

Ewart dispenses with the last reason by means of numerous convincing illustrations of the connexion between the aftershafts of two, and even three generations of feathers in birds of such widely separated families as Casuariformes, Sphenisciformes, Anseriformes, and Galliformes.

The first reason is seen to be equally unfounded from a study of the development of the aftershaft. As the feather grows from tip to base, the barbs of the aftershaft are formed at the same time as the barbs of the shaft, and it is the fusion of these two structures which forms the calamus.

Thus a complete feather must consist of a calamus, shaft and aftershaft, although the latter may appear vestigial owing to the delayed fusion of the barbs of the ventral side of the feather.

VII—SUMMARY

The nestling coat of the duck consists of prepennae, preplumulae and prefiloplumae ; of the goose, prepennae and preplumulae, and of the chick and Passerines (except the House Sparrow) of prepennae.

These feathers represent protoptiles, the mesoptiles having been suppressed.

Nestling and definitive feathers arise in a similar manner from the stratum Malpighii of the skin or the papilla respectively, the stages consisting of:—

- (a) Proliferation of intermediate cells.
- (b) Grouping of intermediate cells to form ridges.

- (c) Grouping of intermediate cells within ridges, forming one median and two lateral plates.
- (d) Appearance of epidermal pigment cells.
- (e) Formation of barbs and barbules from the plates, and their pigmentation.
- (f) Fusion of barbs dorsally forming the rhachis, and ventrally forming the hyporhachis.
- (g) Lateral fusion of rhachis and hyporhachis forming the calamus.
- (h) Withdrawal of pulp from the apex of the feather, simultaneously with cornification, and followed by the formation of feather caps.

The feather sheath consists of the outermost layers of intermediate cells in both protoptiles and teleoptiles, in the former also supplemented by the epitrichium.

Prior to moulting, the feather papilla resumes activity and the tip of the new feather is formed inside the base of the calamus of its predecessor.

From its mode of development, a typical feather must consist of shaft, aftershaft, and calamus.

II—THEORIES OF FEATHER DEVELOPMENT

I—FEATHER DEVELOPMENT

The theories of feather development propounded by DAVIES (1889) for the pigeon, and supported by STRONG (1902a) for *Sterna hirundo* passed unquestioned until LILLIE and JUHN (1932) put forward the concrescence theory. This is an attempt to explain variations in form and pattern of feathers, produced experimentally by the injection of female hormone or by thyroid medication, as due to different rates of growth of individual barbs, and the basis of this theory lies in their conception of the rhachis of a feather as formed by concrescence of the two halves of the "collar".

The "collar" is defined as the Malpighian layer before the commencement of ridge formation, and LILLIE and JUHN further consider the part of the collar in which the bases of the primary ridges end as the primordium of the shaft, which thus has the form of a ring. The shaft itself according to these investigators, is formed by the concrescence of the halves of this ring in the mid-dorsal line (the dorsal point being fixed by the position of the rhachis) and the growth-energy is furnished by growth and multiplication of its cells directed dorsally from the mid-ventral part of the ring. The rate of growth of each barb is so regulated that it is completed by the time the base meets the mid-dorsal line. There is thus no time in development when the barb is not attached to the primordium of the shaft.

DOMM, GUSTAVSON and JUHN (1932) illustrate the concrescence theory by fig. 57.

The diagrams in fig. 57 offer no indication of the method of formation of the aftershaft—an essential part of the feather, however rudimentary, but it may be inferred that the aftershaft arises at the ventral point in the same way that the shaft arises at the dorsal point. A reversal of the "streaming movement" of cells somewhere between the dorsal and ventral points would be necessary to account

for this, giving the so-called "region of plasmatic growth" where barbs pass either dorsally or ventrally. The apparently double origin of the rhachis in these figures may possibly have been imagined from the longitudinal depression which is obvious macroscopically on the ventral side of the shaft. LOWE (1933) certainly regarded the longitudinal furrow in the middle of the ventral surface of Carinate feathers as

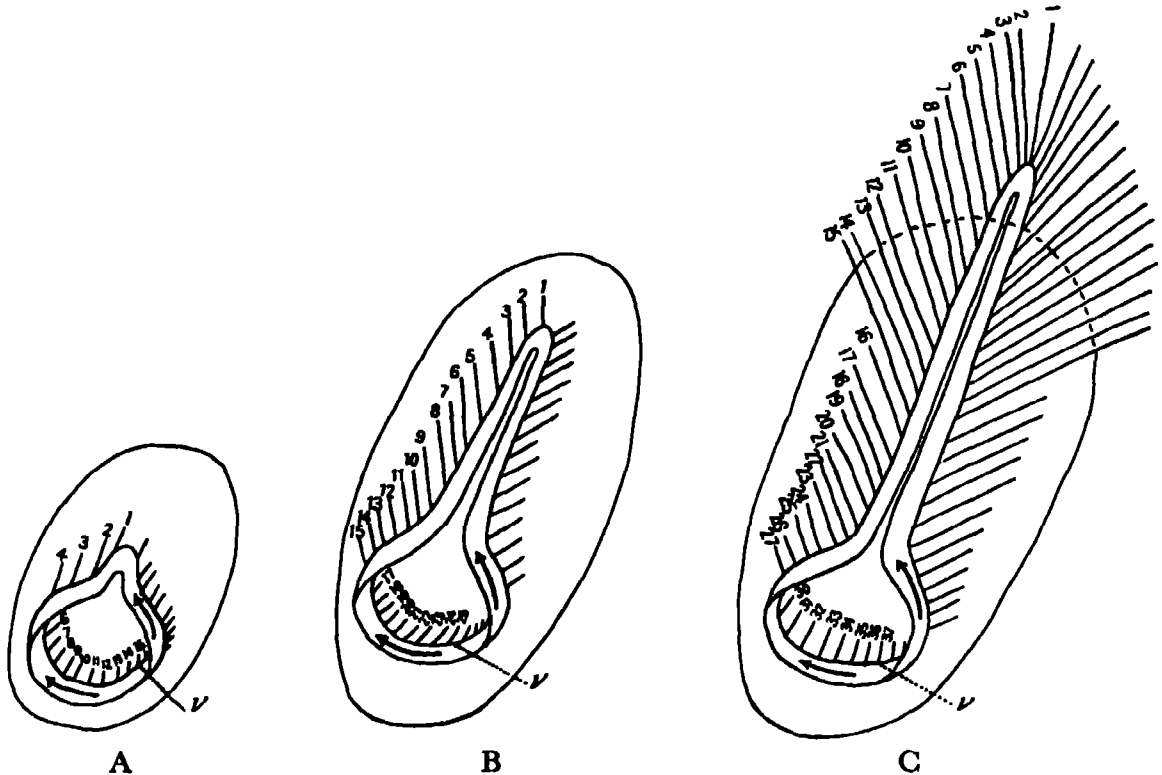


FIG. 57.—The diagrams illustrate the principle of concrescence in the origin of a feather. The two halves arise from a ring of embryonic cells, the "collar" surrounding the base of the feather germ; the ridges or barbs (Nos. 1-15, fig. 57A) arise, each with a separate growth centre from the collar at right angles to it, thus parallel to the axis of the feather germ. The rhachis is formed by a process of concrescence of the continually growing right and left halves of the collar, the levels from apex to base being formed successively, fig. 57A. The forming barbs are carried along with the constantly streaming halves of the collar to their definitive positions at the sides of the shaft with consequent change of orientation. As the series of barbs move dorsally (Nos. 1-15, fig. 57A), new barbs, (Nos. 16-25, fig. 57B), take their origin in the space thus provided at the ventral surface of the collar. At any given time, then, the collar is beset with a series of forming barbs on each side which range in age from the dorsal to the ventral surface of the feather germ and in state of development from the completely formed barbs dorsally, (Nos. 1-25, fig. 57C), to mere apical rudiments ventrally, (Nos. 35, 36, 37, fig. 57C). The order of formation of the shaft is apico-basal and the order of age of the barbs is naturally the same. Similarly in each barb the apex at the margin of the feather is the first formed and the central end attached to the shaft last. Thus there are two time gradients in each feather, from apex to base along the shaft and from margin to centre along the barbs. (From DOMM, GUSTAVSON and JUHN, 1932, p. 638.)

"marking the point where the fusion of the two incurving lateral halves of the feather has not been quite complete" (p. 492). In this case, how would these authors explain the triplication of such depressions in feathers from certain regions which are so conspicuous microscopically, fig. 52, Plate 28.

A more exact representation of the sequence of events is given in fig. 58.

The barbs form simultaneously near the dorsal point at the tip of the feather germ, one of which may be slightly larger than its neighbours, or there may be no difference in size, fig. 58 A. The fusion of barbs to form the shaft, and the similar fusion to form the aftershaft are shown in fig. 58 B, C, and D. Meanwhile, growth has been continuing at the base, so that the whole feather germ has greatly increased in length, but the pulp is still keeping pace with the rest of the feather, fig. 58 E. Fig. 58 F shows that withdrawal of pulp and formation of feather caps has begun, and the bases of the rhachis and hyporhachis are rapidly thickening. This is followed by the lateral fusion of rhachis and hyporhachis, giving rise to the calamus, and marking the site of the superior umbilicus, fig. 58 G. At this time, too, the first formed barbs have broken from the sheath. The series of cross-sections in fig. 56, also illustrate this. The difference between the two theories of feather development is made clear by a comparison of figs. 57 and 59.

LILLIE and JUHN summarily dismiss the theory that the rhachis is formed by fusion of barbs, saying "This is however, incorrect. . . . The two dorsalmost barbs are laid down and become pigmented and keratinized while still separate from one another. After that there can be no fusion except at their undifferentiated bases ; it is at their bases, as a matter of fact, that the shaft proper begins by a fusion of the two half-rings of the collar to which the bases of the first barbs, as well as those of all succeeding barbs have a primary attachment." (Pp. 142-143.)

If successive cross-sections of a feather are taken from tip to base, it will be seen that the two most apical barbs fuse to form the rhachis before cornification commences, and since the feather is growing only at the base, this fusion is really a combination of their growing basal regions. In fact, the process of cornification does not usually begin until several barbs have thus fused. When sections are taken through older feathers in which cornification has proceeded almost to the base, there might appear superficial grounds for such a statement. Actually, the tips of the barbs are keratinized before the bases, but it must be remembered that growth takes place in the reverse direction (*i.e.*, base to tip) and fusion of barbs occurs before cornification has begun. There is thus no justification for the first part of the above quotation.

It has already been pointed out (HOSKER, 1934) that ridge formation is a passive cutting up of the intermediate cells, and these divisions curve round the feather germ in such a way that a ridge which at first lay ventrally, would ultimately fuse with the rhachis at the dorsal point. There is thus no suggestion of a movement of cells, except the early grouping of cells into barb and barbule plates, or of a movement of the two halves of the collar to fuse in the dorsal line.

After refuting DAVIES's conclusions regarding the method of formation of the shaft, LILLIE and JUHN quote him as confirming the concrescence theory. "While

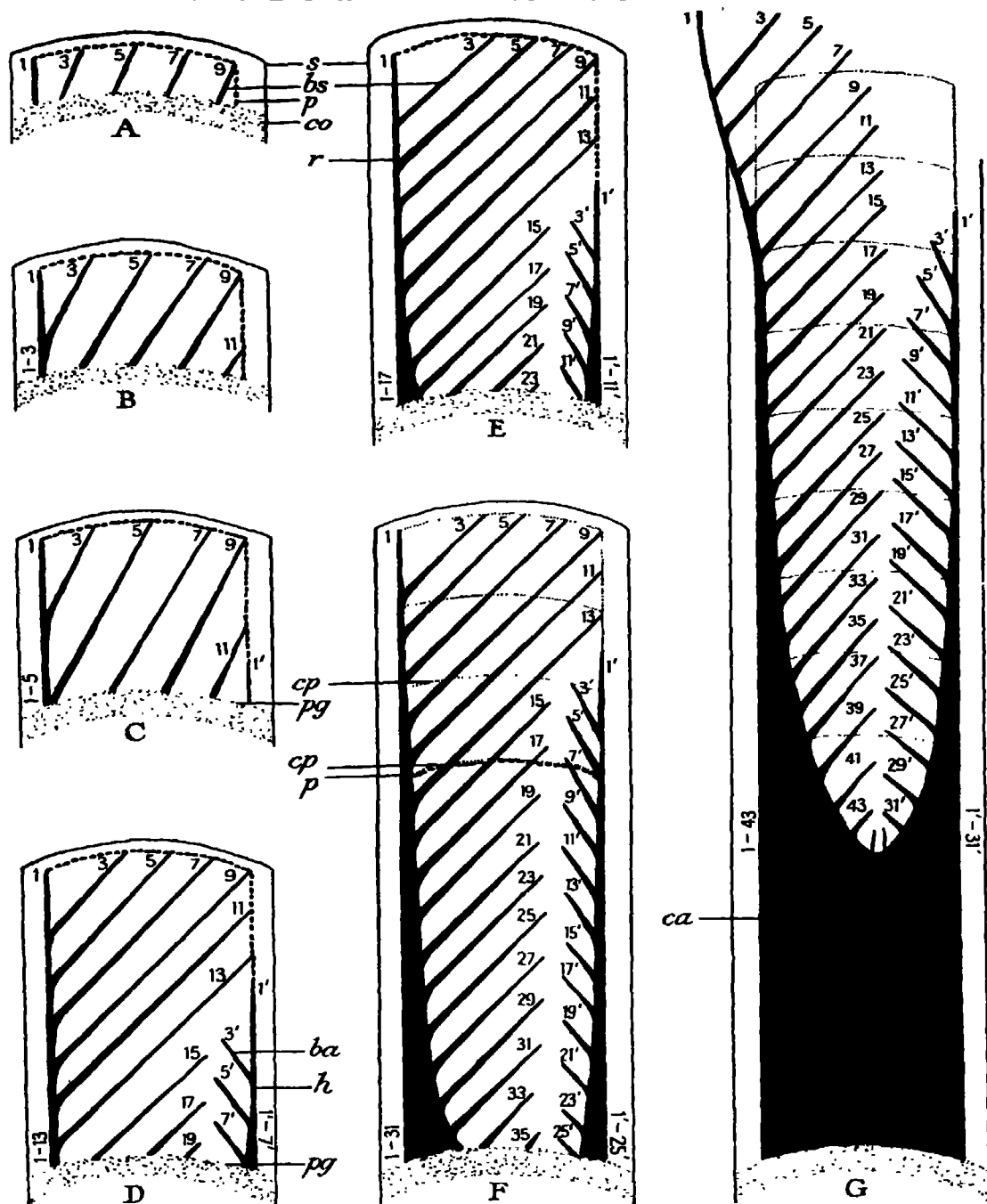


FIG. 58.—Longitudinal diagrams of hemi-sections of feather germs to show the method of formation of a feather according to the fusion of barbs theory. A.—Barbs forming at the tip of the feather germ. B.—Fusion of barbs dorsally to form the rhachis. C.—Appearance of region of "plasmatic growth" where barbs pass either dorsally or ventrally. D.—Fusion of barbs dorsally forming rhachis, and ventrally forming hyporhachis. E.—Thickening of rhachis and hyporhachis, making pulp cavity narrower. F.—Withdrawal of pulp and formation of feather caps. G.—Complete feather, showing the tip having broken from the sheath, the pulp completely withdrawn, and the calamus formed by fusion of rhachis and hyporhachis. These diagrams are of necessity foreshortened and the curvature of the barbs is not shown. The stippled region at the base of each germ represents the collar, where growth (except that due to vacuolation of cells) and pigmentation occur. *ba*—barb of aftershaft; *bs*—barb of shaft; *ca*—calamus; *co*—collar; *cp*—feather cap; *h*—hyporhachis; *p*—pulp; *pg*—region of plasmatic growth; *r*—rhachis; *s*—sheath. 1–43—Barbs fusing to form the rhachis. 1'–31'—Barbs fusing to form the hyporhachis.

in the primitive down the upper border of the *Spule*, to which the rays are fastened, forms a circle, in the definitive feather, in consequence of enormous prolongation of one side, it forms a long drawn-out obliquely placed ellipse, and by the great thickening of this prolongation the upper umbilicus is completely closed. In this manner there arises in the definitive feather a structure, the shaft, which in reality

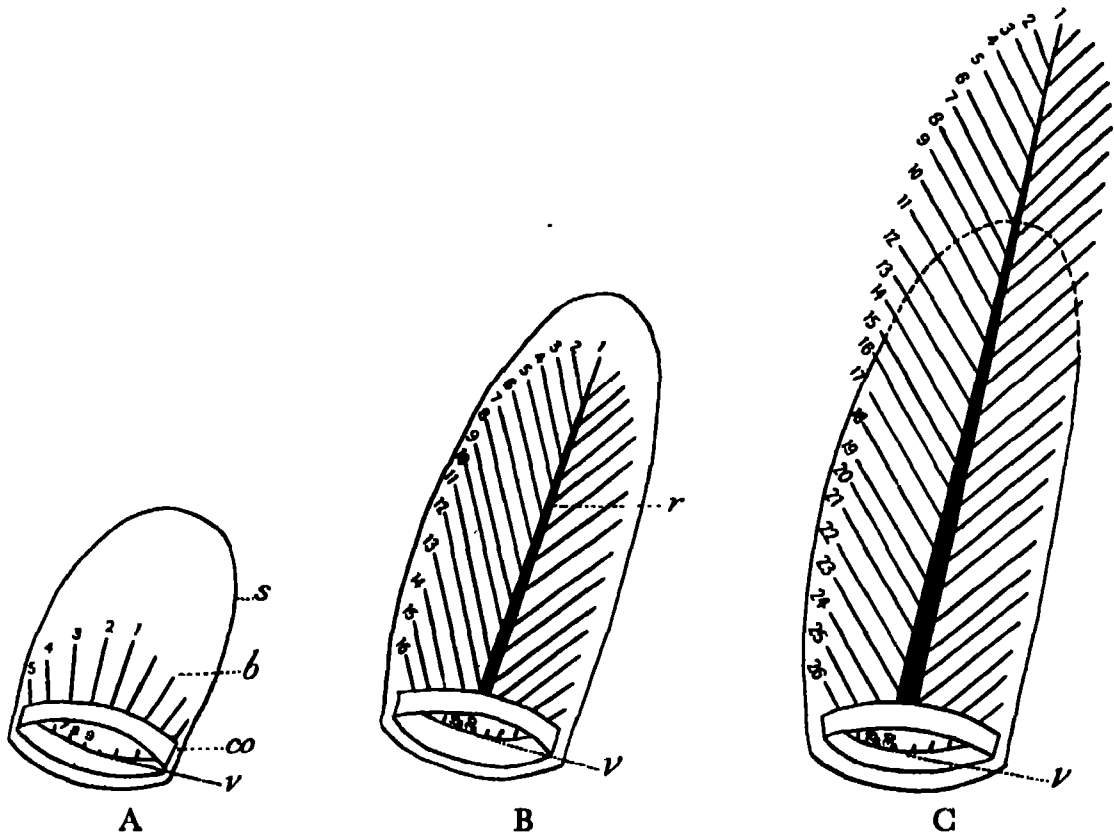


FIG. 59.—Diagrams showing the development of a feather according to the fusion of barbs theory, drawn from the same point of view as fig. 57, and omitting the method of development of the aftershaft. The whole feather arises from a ring of embryonic cells (*i.e.*, the collar) surrounding the base of the feather germ. The barbs, Nos. 1-9, fig. 59A, arise from the collar at an angle of approximately 135° . As more cells are added to the barbs from the rapidly dividing collar cells, it will follow that they will gradually approach the mid-dorsal line, and fuse with the dorsal-most barb or rhachis. This of necessity becomes broader, fig. 59B and C, and takes on the definitive shape of the rhachis. The rhachis cannot therefore be considered as a prolongation of the collar, as is implied by fig. 57, but a structure similar to a barb, and differing only from a barb in occupying the mid-dorsal position. *b*—barb; *co*—collar; *r*—rhachis; *s*—sheath; *v*—ventral.

is only part of the *Spule*, although it makes the impression of an entirely new structure."

The *Spule* LILLIE and JUHN interpret as the collar, but it really represents the calamus, which, as fig. 58 shows, is formed by the fusion of the shaft and aftershaft.

Early workers failed to realize the presence of both shaft and aftershaft in nestling down, however rudimentary they might be, but it has been shown (Part I, p. 173) that a typical feather consists of shaft, aftershaft, and calamus, although the aftershaft may be so small as to be represented by a few barbs only at the lip of the superior umbilicus, *e.g.*, remiges. Thus the *Spule* should really be drawn-out ventrally as well as dorsally to form the basis of a typical feather. DAVIES's statement also quoted by LILLIE and JUHN that "all the parts which bear rays are only specially developed parts of the original *Spule*" may be taken as meaning that the intermediate cell layer (forming *Spule* or collar) is responsible for the entire feather, and thus has no bearing on the method of formation of the shaft.

STRONG, in 1902 (*b*) published an account of an abnormal feather in a hybrid pigeon, in which an attempt at calamus formation was succeeded by the secondary division of this quill-like structure into barbs and barbules. This is difficult to interpret on the concrescence theory of development. If the rhachis arose by concrescence of the two halves of the collar which supposedly forms the primordium of the shaft, the calamus must represent the complete shaft. This admits of no explanation of the method of development of the aftershaft, unless it is assumed to be the ventral side of the shaft. The division of the calamus proximally into barbs in STRONG's abnormal feather can be readily explained on the fusion of barbs theory, as the rhachis and hyporhachis can quite conceivably split apart under abnormal conditions, reversing their method of formation.

This is strikingly demonstrated in the rectrices of young ducklings, fig. 28, Plate 19, where the first generation of nestling feathers has an excessively long calamus, which is continuous with the barbs of the succeeding definitive feather. This condition has also been experimentally induced by feeding thyroid to mature fowls, fig. 55, Plate 28. A condition which occurs abnormally in some birds (*e.g.*, STRONG's pigeon) and normally in others, may be taken as definite proof of DAVIES's statement as to the rhachis consisting of fused barbs.

II—GROWTH-RATE

Regarding the question of asymmetry in feathers, which according to LILLIE and JUHN means "differences in growth-rate on the two sides of the feather germ at some time, or throughout development" (p. 157), there is no evidence that barbs arising simultaneously near the ventral point fuse with the rhachis more quickly on one side of the feather germ than on the opposite side. On the concrescence theory of development this might be so. When, however, ridges are passively cut out from the intermediate cells so that passing down the feather, cells are laid on to the forming barbs from below and gradually nearer to the rhachis, making a barb lying at first near the ventral point ultimately fuse with the rhachis—it is difficult to see how growth-rate can enter into the scheme at all.

ESPINASSE (1934) has recently pointed out some difficulties preventing complete acceptance of the growth-rate theory of LILLIE and JUHN. He considers that

growth-rates on the two sides of the collar can only differ if one of two sets of circumstances holds for that feather germ. "Either (a) the barbs on the two sides must be of different lengths, and the rhachis curved, since one side of it has grown faster than the other; or (b) the feather germ must have an asymmetry of just such a kind and degree as to compensate for the difference in growth-rate and give a straight feather. This asymmetry might be in fact a displacement of the ventral growing point from its theoretical position diametrically opposite the forming rhachis; then the more rapidly growing side would get carried out of the region of growth so much sooner than the more slowly growing side, having the less distance to travel, as to be the same size or even smaller."

Now in certain feathers of the fowl, *e.g.*, remiges, all these conditions are present. The barbs of one side are shorter than on the other; the rhachis tends to be curved away from the shorter side, fig. 54, Plate 28, and also the ventral point is deflected towards the side of the rhachis bearing the shorter barbs, fig. 53, Plate 28.

The curvature of the feather, and also of the rhachis is possibly due to the habit of the bird in holding the wings pressed against the sides of the body. As the follicles are very close together in this position, constant pressure on the developing germ would perhaps cause the feather to assume the curved shape. This, however, does not explain the curvature in other feathers, *e.g.*, the sickle feathers of the male, although in such cases the follicles are invariably crowded. The two facts of (a) the barbs on one side of the vane being shorter than on the other side, and (b) the deflexion of the ventral point are self-explanatory without considering growth-rate.

LILLIE and JUHN emphasize the point that they consider narrowness of the vane to be correlated with rates of growth, but if of two barbs arising simultaneously on either side of the ventral point, one is at a shorter distance from the rhachis than its neighbour, then the vane on that side will be narrower. From the figures given, it is obvious that the right-hand side of the vane in fig. 53, Plate 28, will be much narrower than on the opposite side, while in fig. 52, Plate 28, the vane on the right-hand side will be slightly wider than on the opposite side.

It sometimes happens that the barbs of a feather are not of uniform length along one side of the vane, fig. 54, Plate 28. Occasionally barbs arise which are twice the length of their neighbours. This indicates that the so-called ventral point is not fixed, but in this region of plasmatic growth, a barb which lay nearer to the left side of the feather germ might grow towards the right side of the rhachis. On the concrescence theory, a reversal of the streaming movement of the cells of the collar, carrying with them only one barb centre would be necessary to explain this condition—a hypothesis which is difficult to visualize and which would be still more difficult to prove. Again, it would seem that such a barb would arise on one side of the rapidly growing region, which is supposedly concentrated at the ventral point, and would grow through this region, its rate of growth decreasing at a much later period than in barbs which actually arose at the ventral point. It is difficult to conceive of the growth-rate of such a barb in terms of the diagram of organization of the 12 day feather germ figured by LILLIE and JUHN (1932), p. 129. It is true

that this is for a breast feather, whereas the feather figured here is a secondary flight feather, but such discrepancies have been observed in feathers from all pterylae, although not in such a marked degree.

A recent paper from the Chicago laboratories (JUNH and FRAPS, 1934) states that the "transposition of the definitive feather pattern indicates that the differences in growth-rates in the germ in respect of barb level are relatively small" (p. 1182) and later (p. 1183) with regard to transposing fault bars to the collar, "the apparent differences in barb growth-rates thus arrived at are smaller than are the differences called for by the original curve of barb growth". Thus a more gradual curve is now indicated, but even so, irregular barbs as in fig. 54, Plate 28, do not satisfy the conditions necessary for such a graph.

III—SUMMARY

There are no apparent histological grounds for the concrescence theory of development of a feather, as the rhachis and hyporhachis form through fusion of barbs, and the lateral fusion of these structures gives rise to the calamus.

Asymmetry in feather form arises through the deflexion of the region of "plasmatic growth" nearer to one side of the rhachis, instead of its being fixed diametrically opposite the dorsal point. This results in narrowing of the vane on one side, irrespective of growth-rate in the barbs, and occasionally in lack of uniformity in length of individual barbs.

III—REGENERATION OF FEATHERS AFTER PLUCKING

I—INTRODUCTION

A common method of studying plumage reactions in relation to internal secretions consists of plucking numbers of feathers from different pterylae, before or after the administration of certain hormones. The feathers which are subsequently regenerated are presumed to have grown under the influence of this hormone, and may be expected to show its full effect. In an unplucked bird as the majority of feathers are fully grown and therefore dead structures, the hormone can have little effect. It is generally understood that the annual moult is marked by, and probably the result of a general upset in the metabolism of the bird, and hence such a time of new feather growth is unreliable for the progress of experiments. Although a considerable amount of work has been done on experimental moulting, as contrasted with deliberate plucking (Zawadowsky, 1925; Zawadowsky and Rochlin, 1927) it would be obviously unsuitable to make use of the resultant new growth of feathers for the assay of the effects of hormones.

The method usually adopted, therefore, consists of denuding areas on the birds some time before, or immediately after the experiment is scheduled to begin. On the whole, this appears satisfactory, but actually the results may be deceptive. For instance, it is well known that after plucking, some feathers regenerate more rapidly than adjacent feathers of the same tract. This has been assumed to be due to the follicles of the more rapidly regenerating feathers being nearer the normal period of moult than the more slowly growing feathers (Lillie and Juhn, 1932), but when one regenerating feather may be fully grown before any sign of regeneration in its neighbour occurs, this assumption breaks down. Again, plucking will cause some injury to the papilla, unless the plucked feather is on the point of moulting, and thus the method of replacement of plucked feathers cannot be identical with that of feathers moulted in the ordinary way.

The mechanism of regeneration has not yet been fully worked out. LILLIE and JUHN (1932) sectioned papillae immediately after plucking, and found the epidermis completely lacking at the tip. When some time had elapsed after plucking, the feather germ was seen by these authors to consist of (*a*) an apical zone where the pulp is constantly dying off; (*b*) a middle zone occupied by barb primordia; and (*c*) a basal zone occupied by the collar and without barbs.

It seemed advisable, therefore, to make a detailed study of the interval between the stages noted by Lillie and Juhn, and to compare the formation of the feather regenerating after plucking with the normal replacement already described (Part I, p. 167).

II—MATERIAL AND METHODS

For the detailed study of early stages of regeneration, feathers were plucked from the back and thigh of Rhode Island Red fowls aged 8 weeks, at intervals of 72, 51, 27 and 4 hours before killing. Later stages were obtained by plucking a 16-weeks old fowl of the same breed in the following areas: (1) neck, 8 days; (2) anterior and posterior breast, 7 days; (3) thigh and abdomen, 6 days; (4) anterior back, 4 days before killing. This bird had been addicted to cannibalism, and the posterior back showed various stages of regenerating feathers of unknown age.

In all cases, the skin of the plucked region was fixed in Bouin's solution; feathers embedded in celloidin and wax by the long method (Part I, p. 145); cut from 6–8 μ ; stained in iron haematoxylin (short method) and counterstained in picro-fuchsin.

III—EARLY STAGES

Even in the early stages of regeneration (4–72 hours after plucking) great variety exists between individual germs. All feathers are still deep within the follicle, but occasionally a small plug of cornified material projects from the mouth. This is probably due to profuse bleeding, and cannot be considered as due to the activity of the papilla. Microscopic differences are apparent showing differences in the

amount of healing and regeneration in adjacent follicles, but these are not so pronounced as in the later stages studied (4–8 days after plucking). Whereas in the early stages it is possible to judge the age of regenerating feathers with a fair degree of accuracy, in the later stages it is quite impossible in individual feathers. In the area as a whole, by taking the average lengths of numerous regenerating feathers, the period elapsed since plucking can be ascertained, but not in individual cases. The early stages will therefore be considered in detail, and the later stages without reference to time.

4 hours—Profuse bleeding into the follicle occurs on plucking, which partially obscures the development of the new papilla. The torn base of the old papilla may be seen projecting as an irregular mass of dermal tissue into the cavity of the follicle, fig. 39, Plate 23. There are only traces of epidermis on this papilla. The skin is often torn away from the base of the follicle, and sometimes also from the walls, if the plucked feather happens to be a very young one and therefore with an uncornified sheath still indistinguishable from the sheath of the follicle.

27 hours—Transverse sections through the base of a thigh feather 27 hours after plucking, show the epidermis of the feather and follicle to be fully formed and differing from the normal condition in that a wide, blood-filled space separates them. Very slight traces of ridge formation may be present in the intermediate cells.

In other feathers of the same age, the epidermis of the follicle may be complete, while the papilla is still without an epidermis except for an incomplete ring at the base.

In longitudinal sections of back feathers 27 hours after plucking, the epidermal covering at the base of the papilla is seen to be continuous with the epidermis of the follicle. At the tip of the papilla it is incomplete and broken by blood spaces. Strands of torn tissue project into this space, fig. 38, Plate 23, and may cornify with the follicle sheath if lying in close proximity to it. The epidermis of the papilla becomes constricted below this irregular tip, completely cutting it off.

A plug of cornified tissue is usually present in the mouth of the follicle, and cornified particles line the sides. Examination with the oil immersion lens shows these particles to be chiefly blood cells.

51 hours—The dermal papilla is now more definite, with a complete epidermal covering, and the intermediate cells show signs of grouping into ridges. These incipient ridges are continued below the blood space at the base. Presumably therefore, this region will be pushed into the follicle cavity when more dermal tissue is drawn into the papilla.

Transverse sections of back feathers at this stage show an increase in the amount of cornified tissue in the blood space, fig. 43, Plate 23. It is assumed that the whole of the torn tissue and the blood in the follicle cornify and are carried upwards by the growth of the new feather, being finally pushed out of the follicle and shed with the sheath.

The feather papilla becomes progressively wider in diameter towards the proximal end, while the blood space decreases, until the actual base presents the appearance

of a normal feather, in which the sheaths of both feather and follicle are closely adhering.

In one feather, serial transverse sections from tip to base, show the intermediate cells and cylinder cells projecting inwards and ultimately cutting off a circular feather from the rest of the papilla. Thus the appearance of a feather within a feather is obtained, but this is more marked in the next stage.

72 hours.—The double nature of the feather is continued to the base, where a double crescent of epithelium is seen in transverse section, fig. 41, Plate 23.

Longitudinal sections of back feathers show the whole of the tissue within the previously blood-filled part of the follicle to be cornified, the tip of the small feather cornifying, and the double region still uncornified, fig. 42, Plate 23.

IV—LATER STAGES

The difference in the ages of the newly regenerated papillae is clearly seen in sections of later stages. Some feathers are already extruded from the mouth of the follicle, while others are no further advanced than the 72-hour stage. In a large number of birds plucked for other purposes, this difference is particularly noticeable. Twenty-eight days after plucking, feathers in certain areas are not visible macroscopically, while others are 4 cm or more in length.

The type of development after the third day of regeneration appears dependent on the amount of injury sustained by the papilla. If only slightly injured—whether due to more careful plucking or to the greater age of the old feather (and consequently greater withdrawal of the pulp proximally) the papilla beneath the cornified plug of torn tissue and blood may regenerate a complete new feather. In this case there is little difference between the feather regenerated after plucking, and a feather regenerated after the normal moult (Part I, p. 167). Such a feather will not have barbs extending quite to the tip, as the epidermis there is too thin to give rise to ridges, as described by LILLIE and JUHN (1932). This thin part of the epidermis will be differentiated into two regions, (*a*) a thin layer of intermediate cells which cornify with or without forming incipient ridges, and (*b*) the cylinder cell layer which forms the first cap of the new feather. The whole of this region will eventually be sloughed with the cornified plug when the feather breaks from the sheath.

By far the commoner type of regeneration is that where a transitory papilla is formed after plucking, as indicated above for the 72-hour stage, but intermediate grades are seen. The tip of the new papilla may be split, fig. 44, Plate 24, and the cornified plug extend between the two parts. Regeneration of the epidermis over this split papilla will take place, but no definite barbs are formed in this region. Eventually, the pulp withdraws from the split tip (as in normal withdrawal of pulp) and the intermediate cells cornify, becoming continuous with the sheath. These are ultimately shed, but definite barbs are formed lower down.

If the tip of the papilla is badly torn, the whole of this region is constricted off from a new papilla which forms beneath. Strictly speaking, the "new papilla" is

merely the base of the old one, but since the papilla grows from the base, the torn tip is carried distally, and when the constriction occurs between the tip and the basal region cutting off the former, the latter gives the impression of an entirely new structure, figs. 40 and 42, Plate 23. It sometimes happens that the first papilla is so badly torn that the intermediate cells and pulp are intermingled in an indefinite mass. The intermediate cells, however, appear to possess an inherent capacity for forming ridges, no matter how badly injured or how much they are displaced from their normal position. Fig. 45, Plate 25, shows the dense, partly cornified first papilla with definite signs of ridge formation, the details of which are given in fig. 45a. More proximally, a new papilla is being formed which will actually give rise to the new feather. In extremely bad cases of injury, the tip of the second papilla forms incipient barbs without definite ridge formation. These cornify, but are sloughed with the sheath, and more proximally the true barbs are formed.

The follicle generally assumes a curved shape during regeneration, which is probably due to the distortion caused by plucking, and also to the profuse bleeding into the follicle. This blood is prevented from escaping by the narrow follicle mouth which becomes sealed with coagulated blood. Thus the follicle after plucking seldom retains its original shape, and the new feather follows a curved course in regaining the obliquity to the surface which is characteristic of feathers, fig. 44, Plate 24.

This curvature is seen by the "crease" in the follicle wall which marks off the enlarged cavity of the torn region of the follicle, and forms a definite constriction between the part of the papilla destined to give rise to permanent barbs, and the first papilla or the cornified plug, according to the degree of injury and consequent type of regeneration. This is illustrated by the diagrams of cross-sections in figs. 45-51, Plates 25-27. Fig. 45, Plate 25, is through the tip of the follicle, and shows an indefinite mass of cornifying material within a thick sheath. The sheath of the follicle is also unusually thick, and the distinction between the two sheaths is very obscure. In this cornifying mass attempts at ridge formation may be seen, fig. 45a, Plate 25. Fig. 46, Plate 26, is slightly lower down, and shows the tip of the second papilla; fig. 47, Plate 26, shows the beginning of the crease which marks the region where a constriction will take place between the distal and proximal parts of the second papilla. Above this crease, traces of barbs are to be seen among the intermediate cells of the second papilla. These never form true barbs, although they cornify and become separated from the rest of the epidermis, figs. 48-50, Plates 26-27. The curvature of the whole follicle is well illustrated by the fact that in these serial transverse sections, both first and second, and finally all three papillae are present in the same section, for a certain distance down the follicle. Near the base, the second and third papillae are completely separated by dermal tissue. This is foreshadowed in fig. 51, Plate 27, where the proximal and distal parts of the follicle are separated by intermediate cells.

Regeneration of feathers after plucking, therefore, differs from normal regeneration, owing to the base of the follicle in the former condition having to regenerate

a papilla before the feather can be formed. The completeness of the papilla in normal regeneration, is due to the outer layers of the intermediate cells at the base of the feather germ forming the proximal end of the calamus of the old feather, while the inner ones are differentiated into a protective sheath persisting as a covering to the papilla when the old feather is shed. Regeneration after plucking is further complicated by the extent of the injury sustained by the papilla, and also by the amount of bleeding into the follicle. These two factors are also responsible for the decided curvature in the follicle, through which the new feather has to steer a diagonal course to the surface. As a result of this, the papilla may make several abortive attempts to form a new feather. When the distortion in the follicle and the injury to both follicle and papilla are completely overcome, the regenerated feather is an exact replica of its predecessor.

V—SUMMARY

Regeneration after plucking takes place in the following stages, according to the degree of injury sustained.

A.—*Slight Injury.*

- (1) Cornification of blood and torn tissue in follicle.
- (2) Proliferation of epidermis over torn papilla.
- (3) Formation of new feather from healed papilla.

B.—*Moderate Injury.*

- (1) and (2) As in A.
- (3) Widening of basal part of papilla.
- (4) Formation of second papilla by constriction between narrow, distal part (*i.e.*, first papilla) and wide base (*i.e.*, second papilla).
- (5) Cornification of first papilla.
- (6) Formation of new feather from second papilla.

C.—*Great Injury.*

- (1) and (2) As in A and B.
- (3) Possible grouping of intermediate cells in first papilla to form incipient barbs.
- (4) Formation of second papilla (as in 3B) and formation of incipient barbs from intermediate cells.
- (5) Cornification of first papilla.
- (6) Third papilla constricted off from second papilla.
- (7) Formation of new feather from third papilla.

In all cases, the excess of tissue formed above the actual feather regenerated, is shed with the sheath.

Regeneration of feathers after plucking differs from normal regeneration in that a functional papilla must first be formed, instead of the old papilla giving rise directly to a new feather within the base of the calamus of its predecessor.

My thanks are due to the following : Emeritus Professor GARSTANG, who originally suggested the problems dealt with in Part I of this paper ; Professor SPAUL who kindly revised the manuscript, and the donors of the Ackroyd Memorial Fellowship who provided the means and opportunity for carrying out the investigation.

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DESCRIPTION OF PLATES

Ant., anterior ; *b*, barb ; *ba*, barb of aftershaft ; *bl*, barbule ; *bs*, barb of shaft ; *bv*, blood vessel ; *c*, cortex ; *ca*, calamus ; *cc*, cylinder cell layer ; *co*, collar ; *cp*, cap ; *d*, dorsal ; *de*, dermis ; *e*, epitrichial layer ; *ep*, epidermis ; *ep. f*, epidermis of follicle ; *h*, hyporhachis ; *i*, intermediate cells ; *m*, medulla ; *ml*, Malpighian layer ; *p*, pulp ; *pa*, papilla ; *pc*, pigment cell ; *post.*, posterior ; *r*, rhachis ; *ri*, ridge ; *s*, sheath ; *sc*, stratum corneum ; *v*, ventral ; *pg*, region of "plasmatic growth".

PLATE 16

- FIG. 1—Transverse section of skin from the spinal tract of a 10 days embryo Aylesbury duckling, showing the elongation of the Malpighian cells and the concentration of the dermis.
- FIG. 2—Transverse section through a feather filament of a 16 days embryo Aylesbury duckling, showing the intermediate cells of each ridge grouped together into median and lateral plates.
- FIG. 3—Transverse section through the skin of the spinal tract of an 11 days embryo Aylesbury duckling, showing the elevation of the feather germ with the steeper slope on the anterior side, the whole greatly increased in size from the previous day.
- FIG. 4—Transverse section through the base of a feather filament of a 17 days embryo Aylesbury duckling, showing the aggregation of intermediate cells into ridges, while the cylinder cell layer has not yet passed between them.
- FIG. 5—Detailed drawing of the part of 6 lying between the lines a and b, showing the preliminary stages of pigmentation.
- FIG. 6—Diagram of a transverse section through a prepenna and two preplumulae of a 15 days embryo Khaki Campbell duckling, showing the relative size of the two and the arrangement of the ridges.

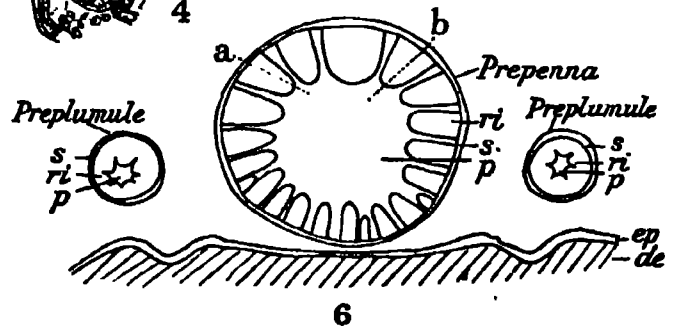
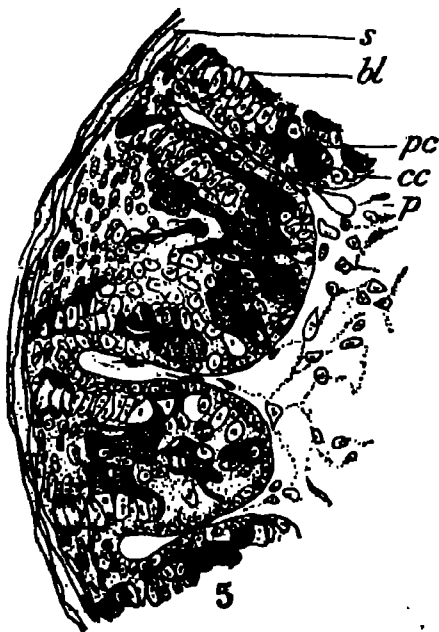
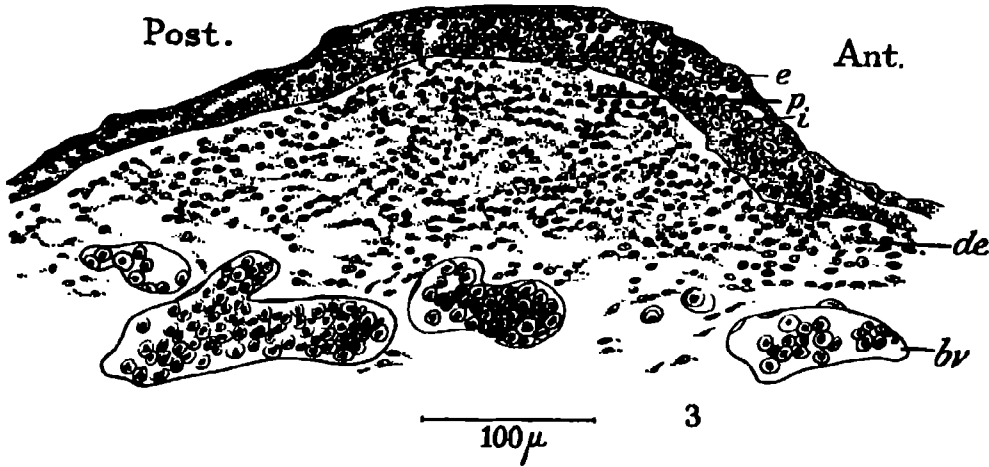
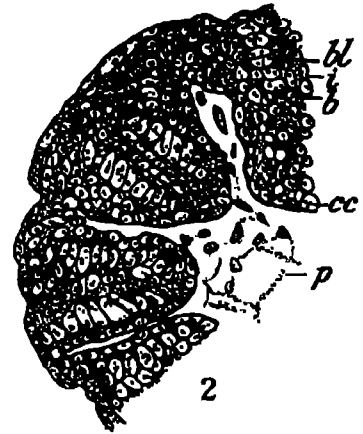
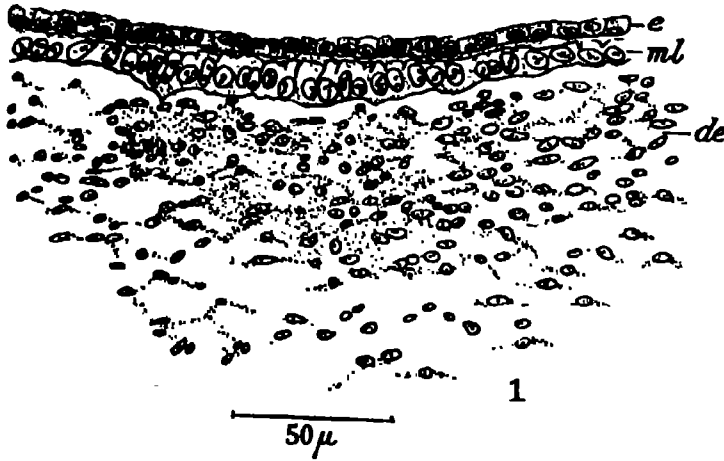


PLATE 17

- FIG. 7—Diagram of a longitudinal section of a 15 days embryo Aylesbury duckling, showing the feather follicle and the continuity between corresponding layers.
- FIG. 8—Detailed drawing of the part of 7 between the lines a and b, showing the differentiation of the intermediate cells of each ridge into barbs and barbules, and the withdrawal of the cylinder cell layer.
- FIG. 9—Transverse section of a filament from an 18 days Khaki Campbell duckling embryo, showing the detailed structure of a barb and the withdrawal of the cylinder cell layer.
- FIG. 10—Diagram of a transverse section near the base of a teleoptile from the wing of a chick (20 days) ; the smaller ridges on the left hand side represent the aftershaft.
- FIG. 11—Transverse section near the tip of a filament of a 20 days chick embryo, showing the crowded arrangement of barbs and barbules, and their appearance after cornification.
- FIG. 12—Detailed drawing of the part between the lines a and b in 10, showing the fusion of barbs to form the rhachis.
- FIG. 13—Detailed drawing of the rhachis and adjacent barbs of the teleoptile of a chick embryo (20 days), showing the typical shape of the barbs and the elongation of the barbule cells.
- FIGS. 14-16—Diagrams of sections taken at the levels marked 14, 15, 16, in fig. 27. 14 showing the decrease in size of the barbs from the rhachis towards the ventral point. Fig. 15 shows the barbs of the shaft, the barbs of the aftershaft not being present. Fig. 16—Section through the junction of a protopile and a teleoptile. The darkened barbs belong to the teleoptile and the light ones to the protopile.

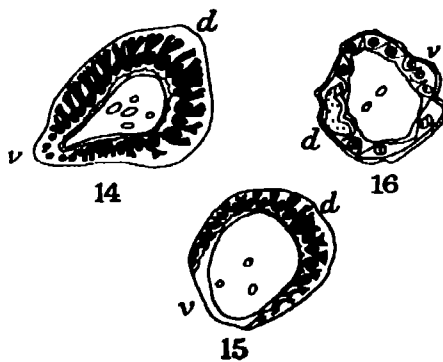
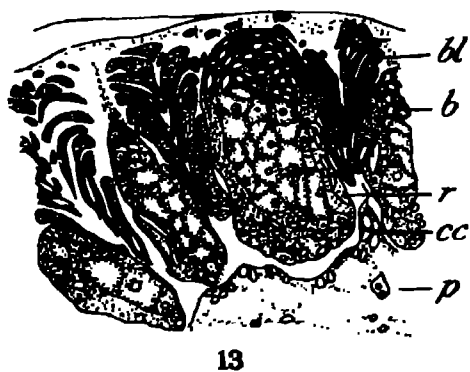
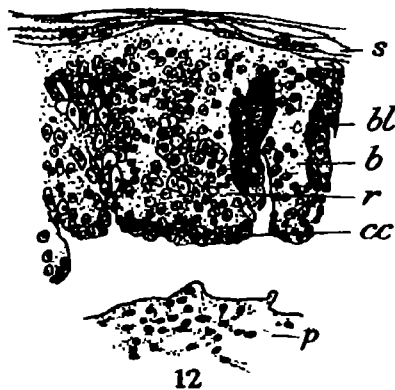
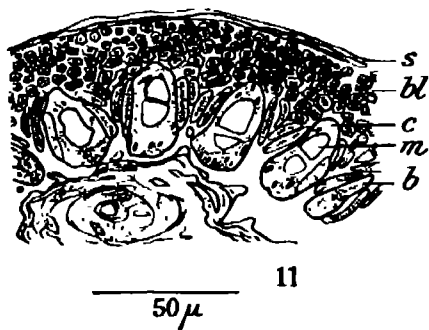
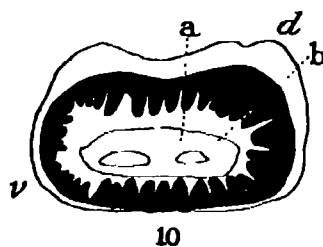
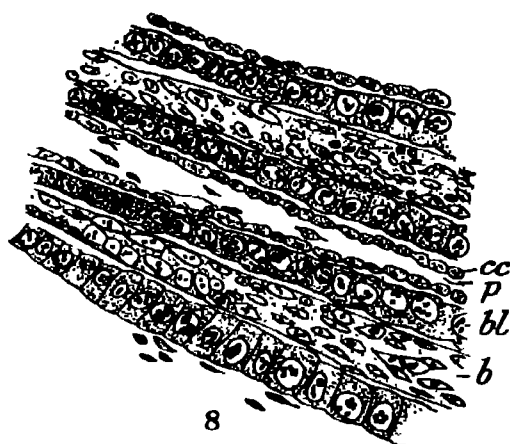
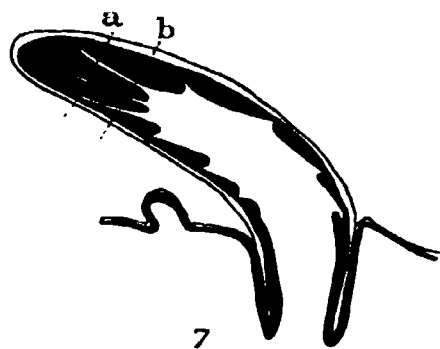
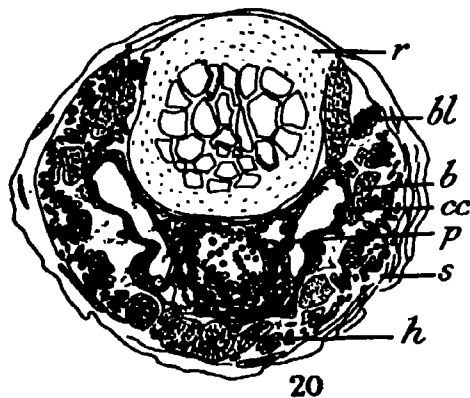
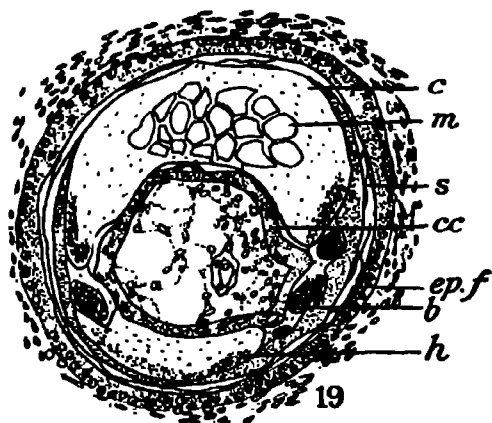
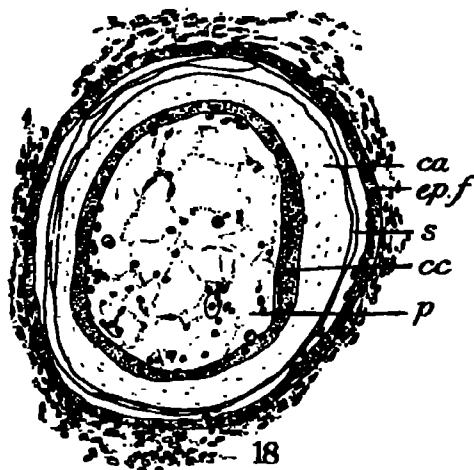
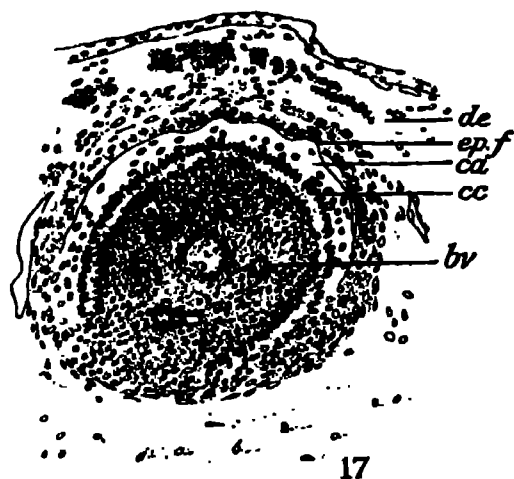


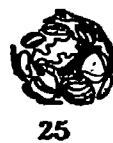
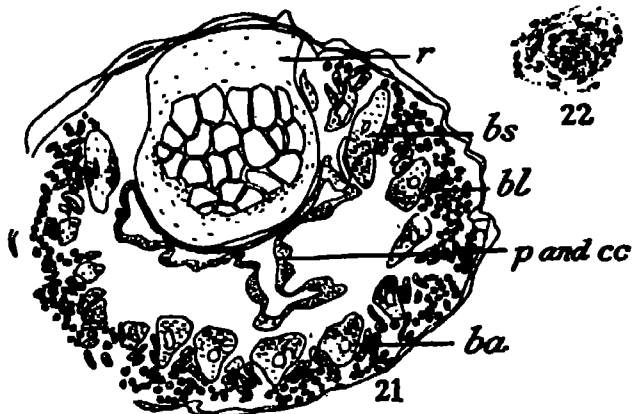
PLATE 18

FIGS. 17-21—Transverse sections taken at different levels of a protoptile of a 24 days Khaki Campbell duckling embryo.

FIGS. 22-26—Transverse sections taken at corresponding levels of a preplumule of a 24 days Khaki Campbell duckling embryo.



100 μ



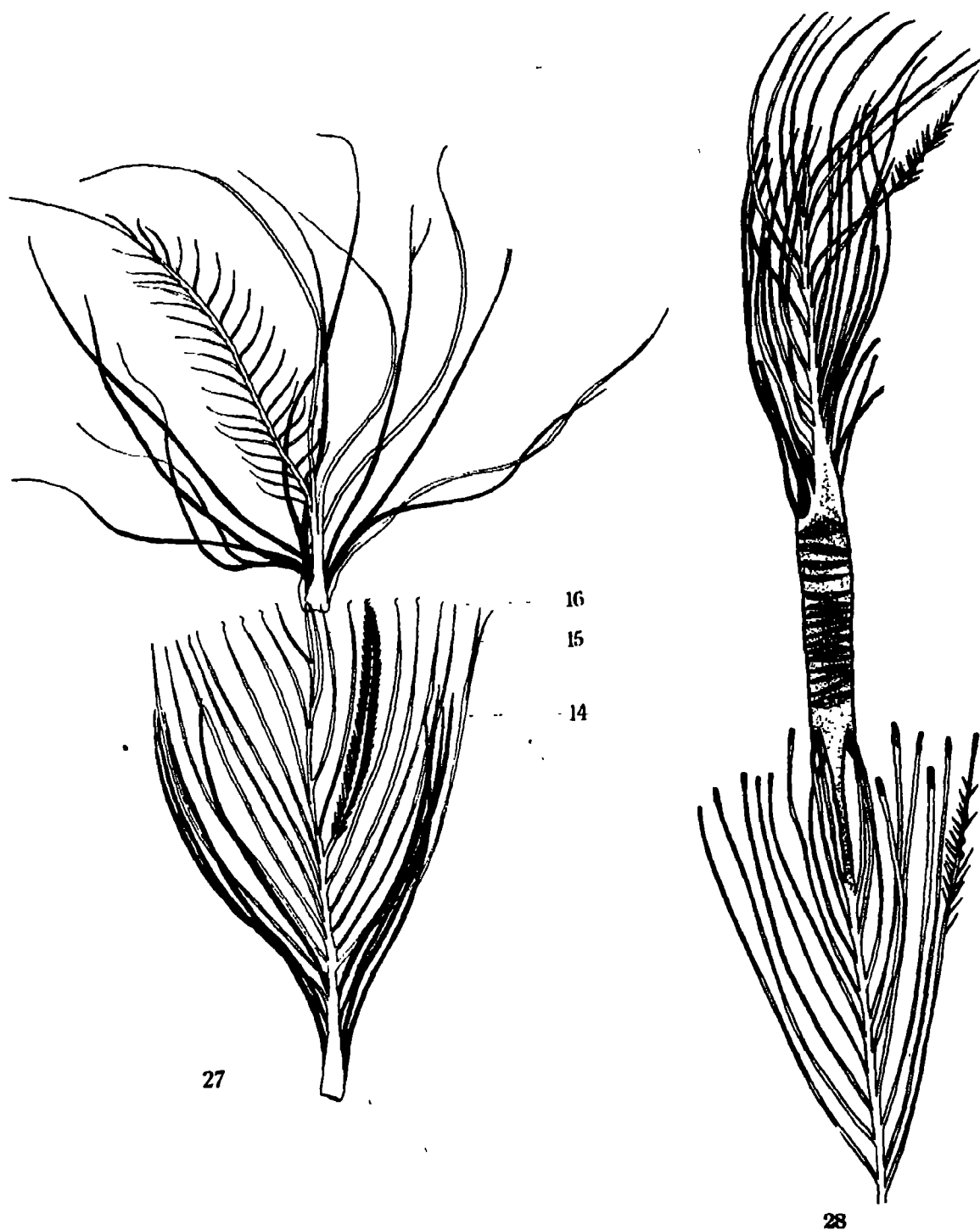


PLATE 19

FIG. 27.—Protoptile and teleoptile from the wing of a day old chick.

FIG. 28.—Protoptile and teleoptile from the tail of a 6 weeks old Khaki Campbell duckling.



PLATE 20

FIG. 29—Barbules from (a) prepenna of chick ; (b) tip of remex of fowl ; (c) prepenna of Chinese gosling ; (d) prepenna of domestic duckling ; (e) plumule of wild duck ; (f) plumule of Chinese goose.

PLATE 21

- FIG. 30**—Diagram of a transverse section of a back feather of a 2 months old Rhode Island Red fowl.
- FIG. 31**—Detailed structure of the region of “plasmatic growth” in fig. 30 where barbs pass either dorsally or ventrally.
- FIG. 32**—Detailed structure of part of fig. 30, showing the fusion of a barb with the rhachis.
- FIG. 33**—(a) Detailed structure of the barb and barbules from one ridge of a tail feather of a 2 months old Rhode Island Red fowl ; (b) Two barbs of fig. 33a under high magnification.

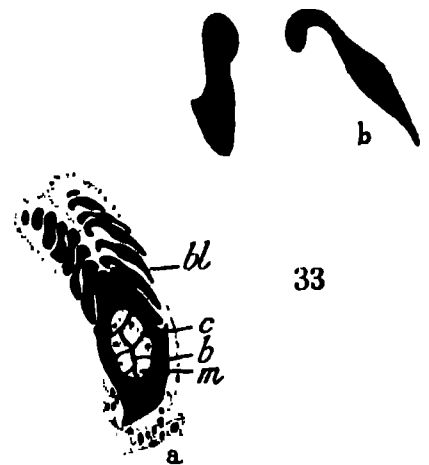
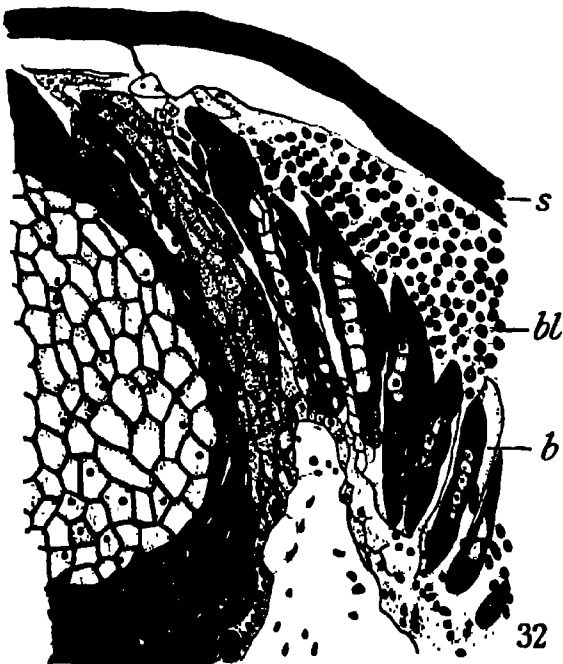
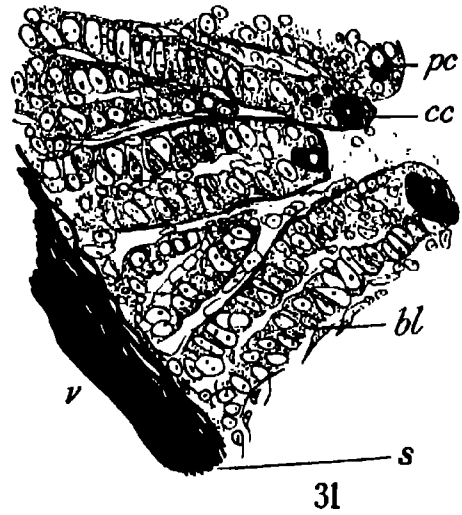
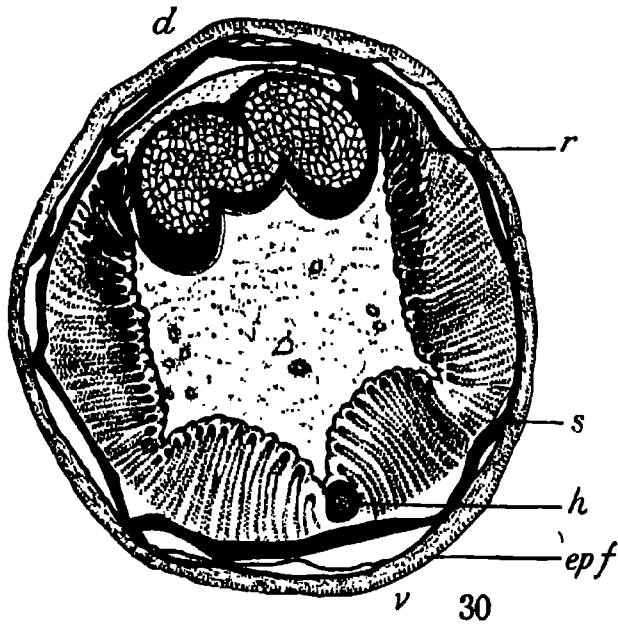


PLATE 22

- FIG. 34**—Diagram of a longitudinal section through the tip of a regenerating feather, showing the feather caps (10 weeks old Rhode Island Red, *tectrice majores*).
- FIG. 35**—Drawing through the base of the feather shown in fig. 34, showing the continuity between intermediate cells round the base of the calamus of the feather about to be shed.
- FIG. 36**—Diagram of the feather papilla shown in detail in fig. 35.
- FIG. 37**—Drawing of a longitudinal section through a developing feather cap in the tail feather of a Starling.

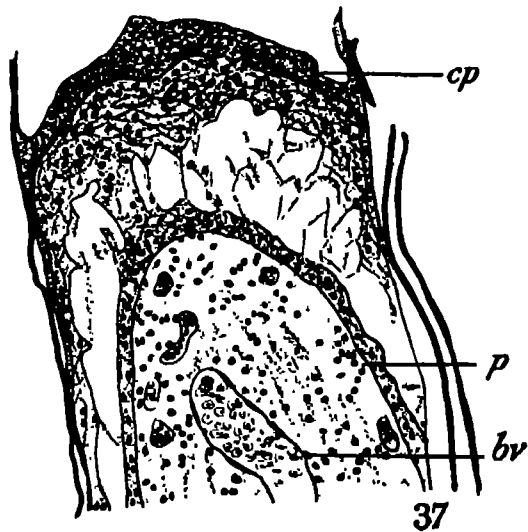
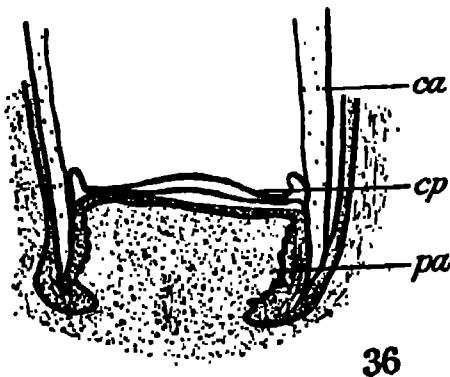
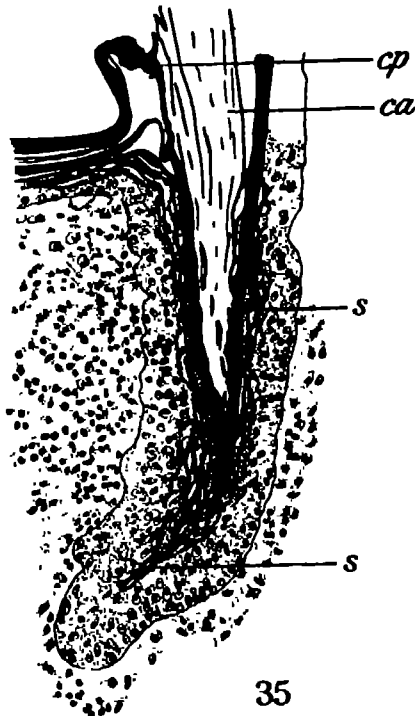
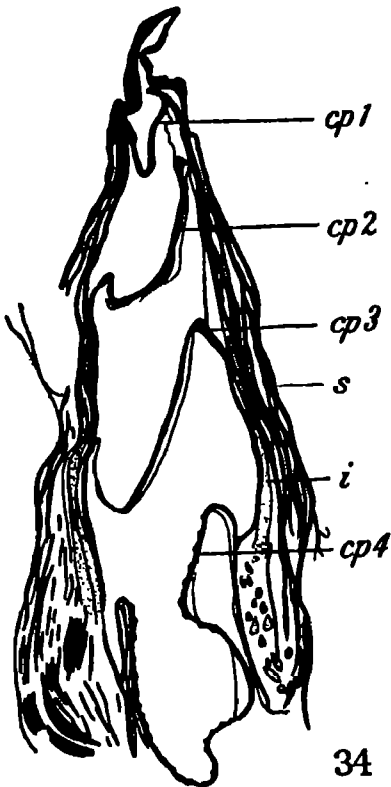
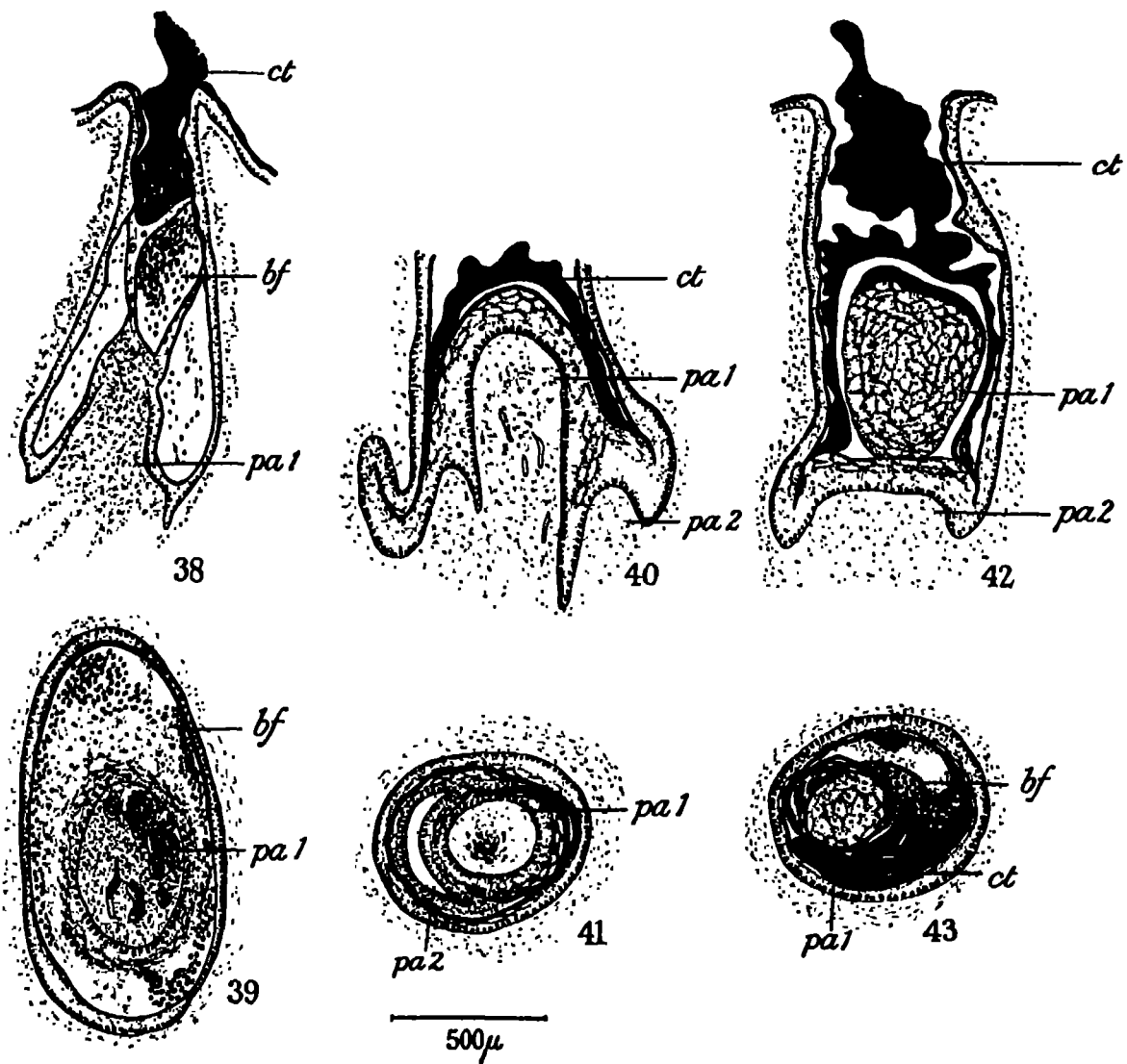


PLATE 23

- FIG. 38—Longitudinal section of 27 hours regenerating back feather.
FIG. 39—Transverse section of 4 hours regenerating back feather.
FIG. 40—Longitudinal section of 51 hours regenerating thigh feather.
FIG. 41—Transverse section of 72 hours regenerating back feather.
FIG. 42—Longitudinal section of 72 hours regenerating back feather.
FIG. 43—Transverse section of 51 hours regenerating back feather.

bf, blood in follicle ; *ct*, cornified tissue ; *sf*, sheath of follicle.



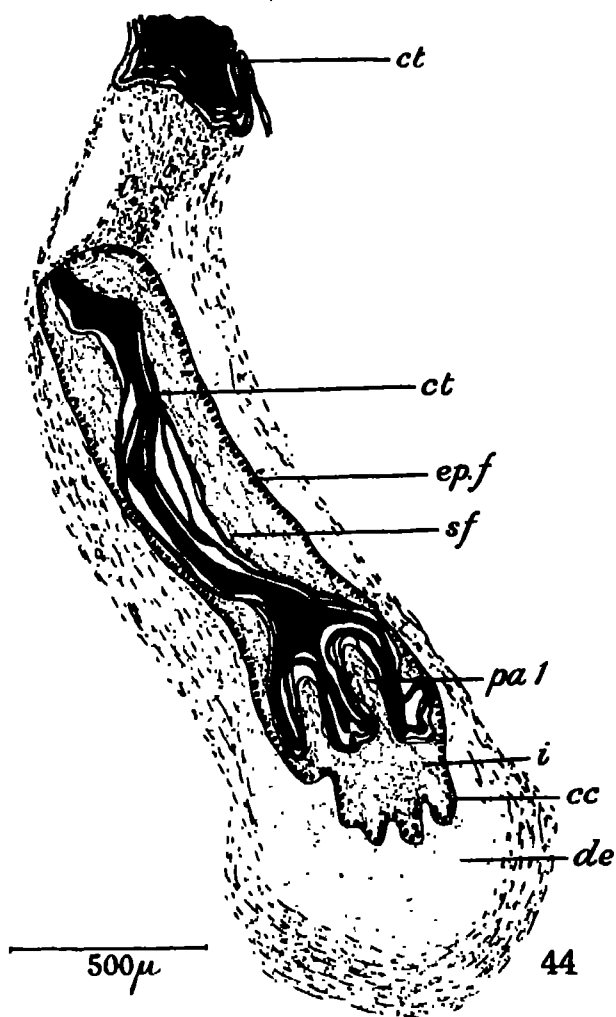


PLATE 24

FIG. 44—Longitudinal section of 6 days regenerating thigh feather.

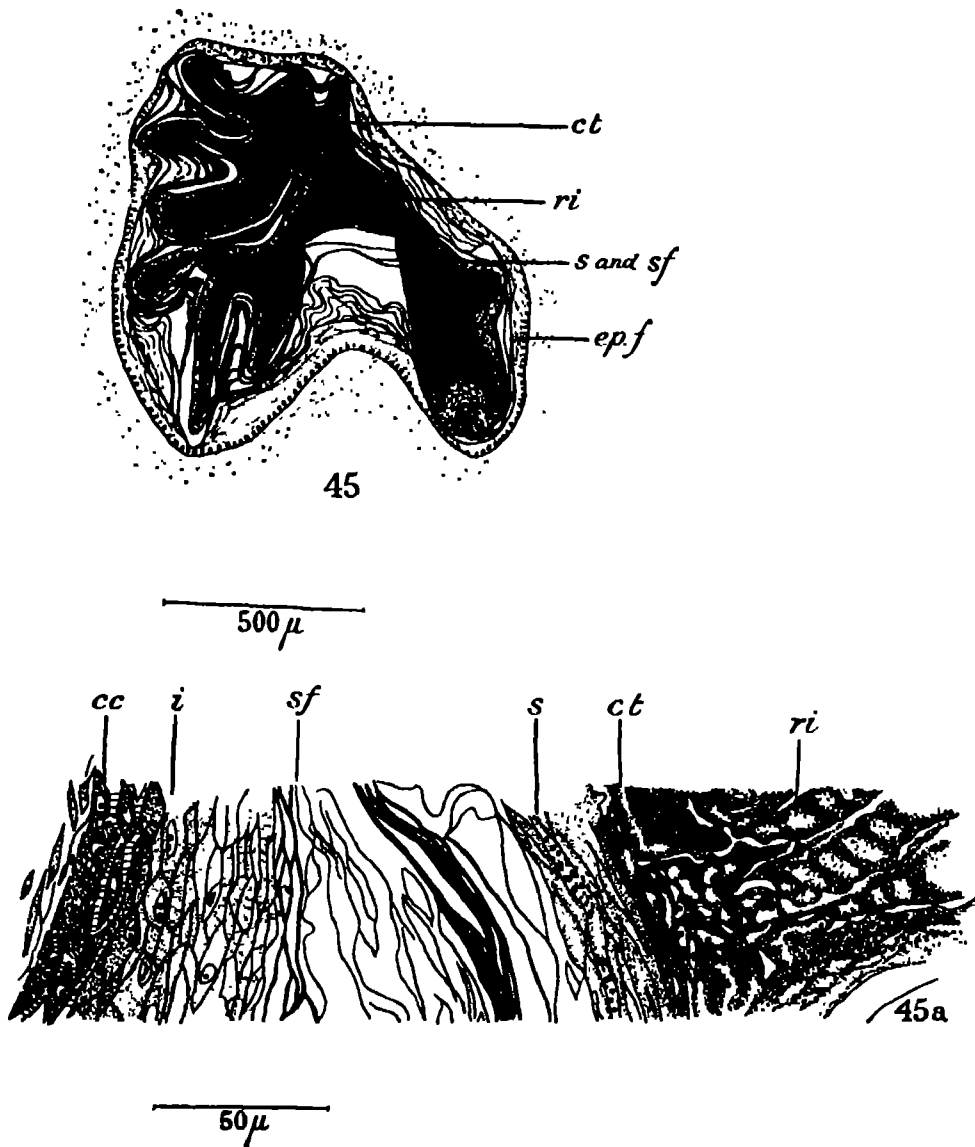
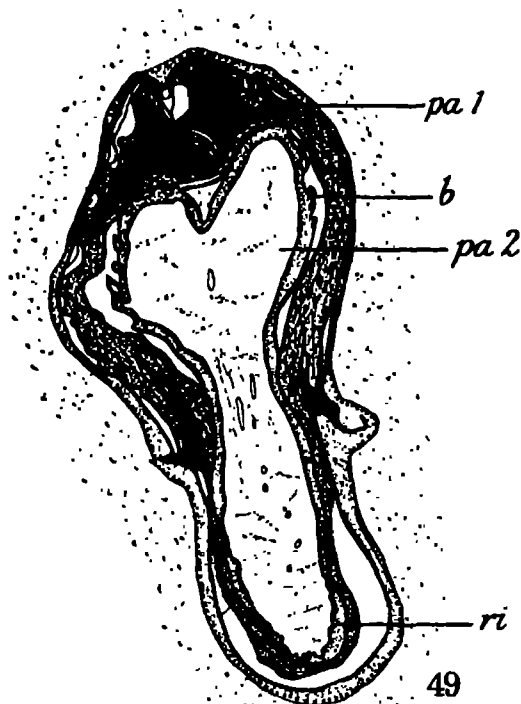
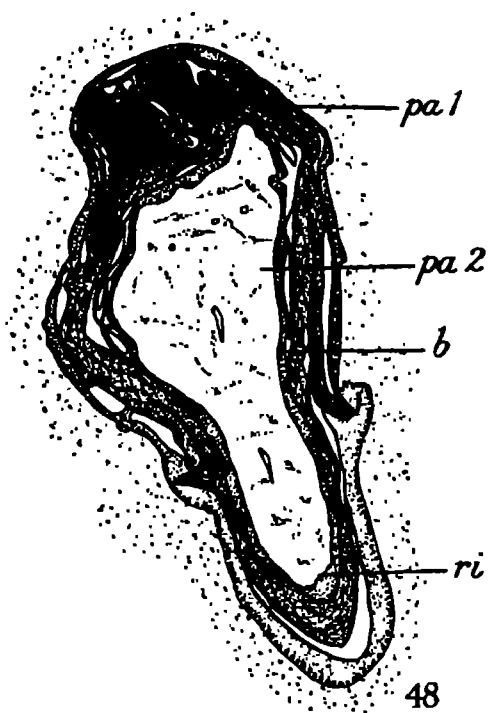
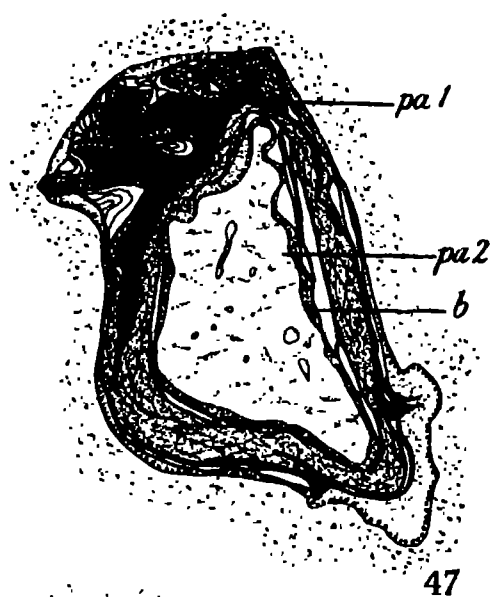
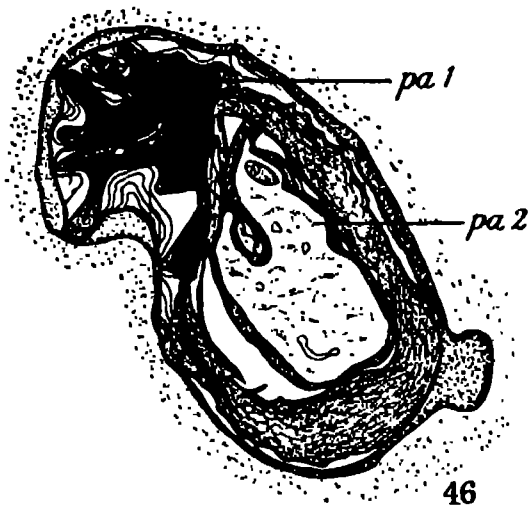


PLATE 25

FIG. 45—Transverse section of tip of 7 days regenerating posterior breast feather.
FIG. 45a—Drawing of part of fig. 45 under oil immersion lens.

PLATE 26

FIGS. 46-49—Transverse sections of same feather as in fig. 45, from tip to base.



500 μ

PLATE 27

FIGS. 50 and 51—Transverse sections of same feather as in fig. 45, from tip to base.

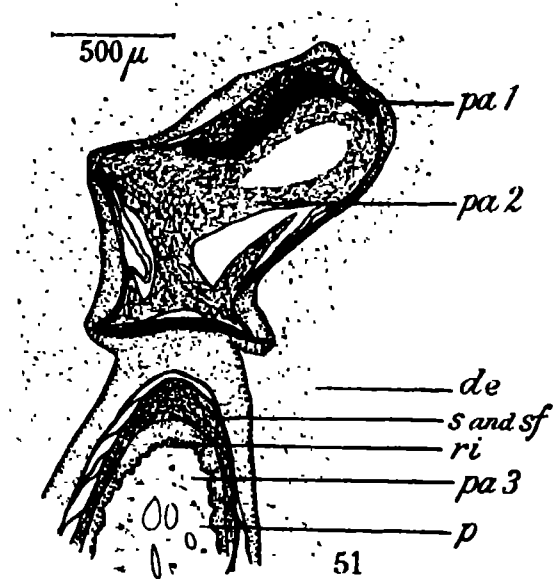
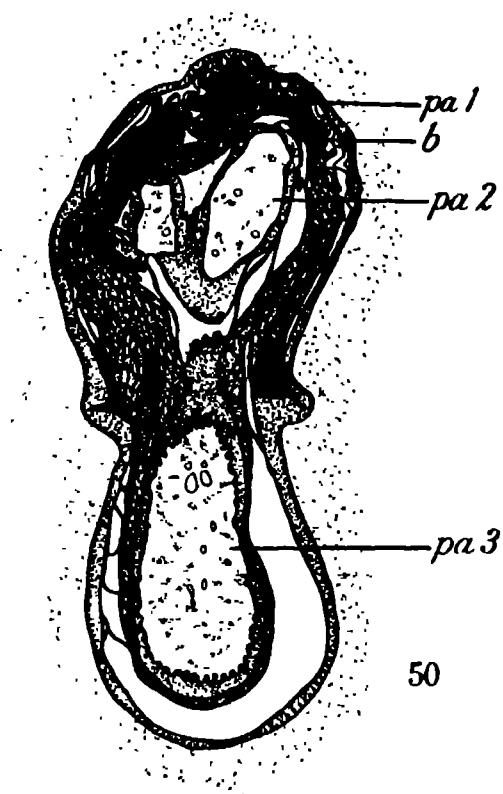


PLATE 28

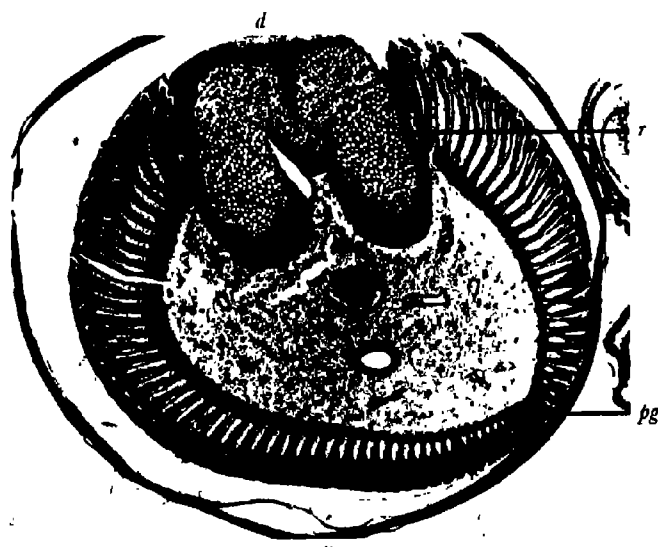
- FIG. 52—Microphotograph of transverse section near the base of a thigh feather of an 8 weeks old Rhode Island Red fowl, showing the deflexion of the region of plasmatic growth nearer to the left side of the rhachis.
- FIG. 53—Microphotograph of transverse section near the tip of a primary of an 8 weeks old Rhode Island Red fowl, showing the deflexion of the region of plasmatic growth nearer the right side of the rhachis.
- FIG. 54—Photograph of a secondary from the wing of a 6 weeks old Rhode Island Red fowl, showing (a) asymmetry in the width of the vane ; (b) curvature of the rhachis towards the wider side of the vane ; and (c) unequal length of adjacent barbs.
- FIG. 55—Microphotograph of the junction between two generations of feathers from the back of a Rhode Island Red fowl fed 30 grams thyroid, showing continuity between barbs of the new and the calamus of the old feather.



52



54



53



55

VI—The British Silurian Rugose Corals with Acanthine Septa

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(Communicated by W. D. LANG, *Sc.D.*, *F.R.S.*—Received January 7, Read May 21, 1936)

[PLATES 29 and 30]

INTRODUCTION

This paper seeks to elucidate the phylogeny of those British Silurian Rugose Corals which have acanthine septa, *i.e.*, whose septa can be seen by the naked eye each to consist of a row of spines. Laboratory studies have been supported by field work, with the object of discovering the range of the species in time and space, and the conditions of life under which they lived ; and of determining whether the evolution of any character received impetus from a change in such conditions. It would appear that ancestors of that Rugose coral fauna which became dominant in the Wenlock, entered the British area in Llandovery times. The Streptelasmoid fauna characteristic of the Ordovician first shows intermixture with Wenlock forms at the top of the Lower Llandovery beds of Llandovery, where *Calostylis* occasionally occurs. In the Upper Llandovery the Calostylidae increase, and the Acanthocyclus and primitive members of the *Pycnactis-Phaulactis* lineage appear. From *Pycnactis* a large proportion of the Wenlock and Ludlow Rugose corals descended. *Streptelasma* seems to have died out at the top of the Wenlock. In the shaly facies of the British Wenlock-Ludlow, a characteristic fauna of *Pycnactis mitrata*, *Syringaxon siluriensis*, *Acanthocyclus* spp. and *Spongophylloides* spp. slowly evolved. When more calcareous conditions appeared, this was enriched by new forms. With the reef facies of the Wenlock limestone, a large number of new species appeared. The British area is a most interesting, though not the most useful, area for the study of the evolution of the Silurian Rugose corals, for here reef conditions were confined to the top of the Wenlock. It has to be decided which new forms evolved from species already present in the area, and which came in from other areas with the reef conditions. Such migrations can only be proved by reference to Scandinavia and N. America, where reef conditions occurred throughout the Silurian. In the present study such migrations cannot be taken into account, because field work has so far been confined to the British area.

All available evolving characters have been studied at each stratigraphical horizon. It is found that phylogenies based on only one evolving character are at length reduced to absurdity, and, in order to appreciate the extremely complicated and interwoven processes of evolution, we must study all possible characters at all possible stratigraphical horizons.

The importance of septal structure as an evolving character is shown here. Workers on Rugose corals have been slow to appreciate the value of septal structure in systematic and evolutionary studies, but in the author's experience this character is of paramount importance in Silurian and Devonian families.

It is therefore appropriate to preface this paper, which gives the first results of a long research on septal structure and phylogeny, with a summary* of our present knowledge of the microscopic structure and formation of the Rugose corallum—a knowledge which we owe to OGILVIE's invaluable researches, amplifying those of VON HEIDER (1882), VON KOCH (1882, *a, b*), and PRATZ (1882). It should be noted that the structures described in this paper concern the coral skeleton *after it has been*

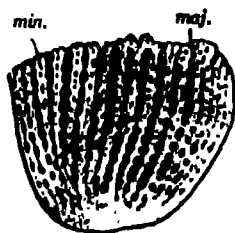


FIG. 1.—A mould of a calice, representing the base of a polyp, which formed acanthine septa. Compare with Hill, 1935, figs. 2A–C on p. 485. The conical invaginations for each trabecula are shown separate in the minor septal series (*min.*), and usually coalesced laterally in the major septal series (*maj.*).

secreted. In this, the controversies of VON KOCH and BOURNE, on the one side, and VON HEIDER and OGILVIE on the other, on the *manner of secretion* of the skeleton are ignored, as irrelevant to the subject-matter of the paper; as is, also, the presence or absence of any organic matter remaining in the skeleton. The microscopic structure of the stony skeleton alone is considered, and here agreement is reached with all the above-mentioned authors who describe this massive skeleton as ultimately built of crystalline fibres of calcium carbonate. It has been pointed out by a friendly critic that the term "fibres" is misleading, as suggesting pliant organic structure, and not rigid crystalline structure. This is strictly true. But since "fibres" has hitherto been generally used by the most eminent authorities (*e.g.*, BOURNE and WAYLAND VAUGHAN); since no other term is readily available—"crystalline fibres" or "fibre-like crystals" are unwieldy terms for structures mentioned on nearly every line; and since the use has a parallel among mineralogists (who describe the minerals "fibrous calcite", "fibrous gypsum", etc.), "fibre" is retained as a descriptive term for the calcareous bodies, bundles of which build up the massive coral skeleton.

Inseparable from studies on the structure and formation of the septa (the chief vertical skeletal elements), are studies on the structure and formation of the horizontal skeletal elements (tabulae and dissepiments), and studies on the thickening of the skeletal elements.

Analogy with Recent corals shows that the Rugose corallum is an exo-skeleton, laid down by the ectoderm of the base of the polyp. It consists of vertical and horizontal skeletal elements enclosed proximally and laterally by a sheath of epitheca. At its distal end is an open cavity, the calice, and a mould of the calice is an image of the base of the polyp (fig. 1). The septa were laid down in radial invaginations in the base of the polyp, and the tabulae and dissepiments were formed from the uninvaginated parts.

* The summary may be supplemented by reference to the author's "British Terminology for Rugose Corals," 'Geol. Mag.', pp. 483–8 (1935).

Microscopic investigation of Rugose corals shows that, as in Hexacorals, the tissue of the skeleton (sclerenchyme) is built of calcareous fibres ; but, whereas in the horizontal skeletal elements the fibres are all parallel and arranged at right angles to the surfaces of the plates, in the septa they are grouped together to form spines (trabeculae) from the axes of which they radiate upwards (fig. 2). In most Rugose corals the trabeculae are arranged in single series, so close together that they form a lamellar septum, but sometimes they are large and separate, at least at the distal edge, so that the septum is acanthine.

Let us now investigate more closely the relations between the exo-skeleton and the ectoderm of the base of the polyp. Since all the species dealt with in this paper have acanthine septa, we shall take as example an acanthine septum in which each spine is a simple trabecula.

The calcareous fibres are always laid down at right angles to the surface of the

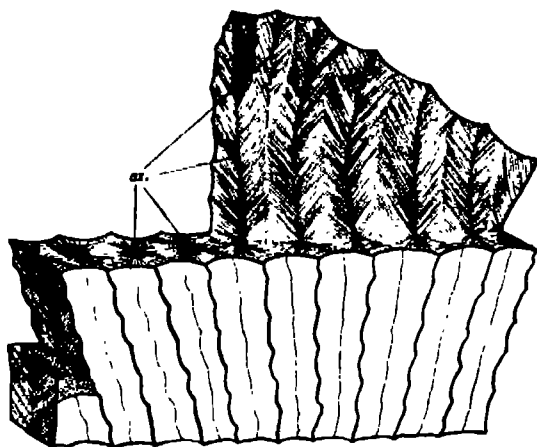
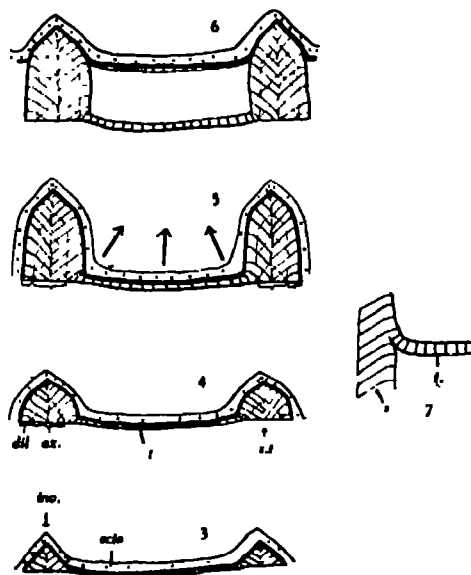


FIG. 2—Diagram showing Trabeculae in a Septum of *Galaxea* (after Ogilvie). Ax., axis of trabecula.

ectoderm which secretes them. Thus, a tabula or a dissepiment consists of parallel calcareous fibres arranged at right angles to its surface. The trabecula is more complicated. It is begun in a point-like invagination (fig. 3). Since this is conical, the fibres laid down by the ectodermal surface will radiate upwards from an axis of calcification. Secretion of fibres is most copious at the apex of the trabecular invagination, and thus the trabecula grows upwards. The invagination deepens and the stretched sides may then add fibres to those laid down by the cone. These lateral fibres approach the horizontal, as the stretched sides approach the vertical. The stretching upwards of the radial series of invaginations causes stresses to develop in the base of the polyp, tending to pull the polyp away from the skeletal floor which it has laid down. The stress is relieved at the critical point by a release and immediate upward movement of the uninvginated base, which continues its secretion at a higher level, forming new horizontal elements. Thus the periodic relief of the stress caused by the rapid growth of the trabeculae causes the deposition

of horizontal skeletal tissue to be discontinuous in space, although there is never any cessation in the secretion of fibres.

Since the ectoderm of the base of the polyp is continuous, the calcareous fibres forming the tabulae and dissepiments are not sharply separated from those laid down by the stretched *sides* of the trabecular invagination; but they are discontinuous with the *axial* calcareous fibres of that part of the trabecula against which they lie (fig. 7), for such were laid down earlier in the apex of an invagination.



FIGS. 3-7—Diagrams showing formation of Septa and Tabulae.

FIGS. 3, 4, 5, and 6 show successive stages. *Ecto.*, layer of ectoderm secreting fibres at the base of the polyp; *inv.*, invagination for the formation of the Trabeculae of the septum; *s.t.*, septa trabecula; *t.*, tabula; *ax.*, fibres secreted by the top of the invagination; *dil.*, fibres secreted by the sides of the invagination. Deposition of fibres is more rapid at the apices of the invaginations than along the uninvginated part of the base of the polyp, and the septa grow upward more quickly than the tabulae. Stresses are thus induced in the base of the polyp. The directions in which the base of the polyp jumps to relieve these stresses are shown by the arrows.

FIG. 7—Shows the fibres of a tabula (*t.*) which began forming later than the part of the septum (*s.*) against which it abuts (*see text*).

In many acanthine septa the distance between the trabeculae is very small, and the proximal parts of the trabeculae rapidly come into contact, squeezing out the intervening flap of polyp. In such cases the septum is lamellar except at its distal edge, and lies in a radial invagination with a crest of small pointed invaginations, one for each individual trabecula.

The species dealt with in this paper belong to the genera *Palaeocyclus*, *Acanthocyclus*, *Tryplasma*, *Cystiphyllum*, and *Cantrillia*. I have confined myself to British forms because hitherto it has been practicable to study only these in the field; but

accordingly I have experienced the disadvantage that, where a species has arisen outside British waters and migrated into them later, its origin cannot be argued from the British deposits, and for the present must remain unknown.

From the evidence available, it is considered that the genera mentioned above form a cognate group, which may be referred to as the Acanthocyclusidae. They all possess a septal structure and a type of horizontal tissue unknown in other corals from Britain, and have not that relation between minor septa and dissepiments which is characteristic of the rest of the Rugosa (HILL, 1935, pp. 508-9). *Palaeocyclus* in all probability gave rise to *Acanthocyclus*; species of *Tryplasma* have undoubtedly repeatedly arisen from species of *Acanthocyclus* and are to be regarded as genomorphs (see SMITH and LANG, 1930, p. 179; 1931, p. 86) of *Acanthocyclus*. The evolving characters were shape, ornament of the epitheca, rejuvenescence, increase of the corallum, septal structure, and horizontal tissue. *Cystiphyllum* and *Cantrillia* share the family characters, but their phylogenetic relation to the other members cannot yet be given.

The following section contains a detailed description of species, and gives the evidence which concerns the relations of species. This evidence is summarized in the conclusion, where also an analysis is given of the results bearing on the structure of the septa, tabulae, and dissepiments, and their relation to the soft parts; and there is also given an outline of the evolution of the various characters in this group.

The material used in the preparation of this paper is in the Sedgwick Museum, Cambridge, except for a few specimens from the British Museum (Natural History) indicated by the letters B.M. The letters S.M. refer to the catalogue numbers of individual specimens in the Sedgwick Museum.

MORPHOLOGICAL DESCRIPTIONS

Genus *Palaeocyclus* EDWARDS and HAIME

Palaeocyclus EDWARDS and HAIME, 1849, p. 71.

Palaeocyclus; LANG and SMITH, 1927, p. 455.

Palaeocyclus porpita (LINNAEUS). (Figs. 8, 11, 14, 18, 26, and fig. 36, Plate 29.)

Madrepora porpita LINNAEUS in FOUGT; (*Madrepora simplex convexa* LINNAEUS 1745, p. 19, fig. v.)

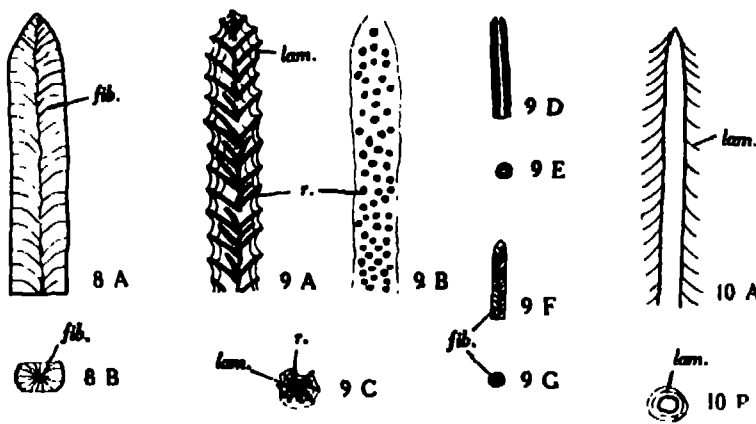
Palaeocyclus porpita (LINNAEUS); LANG and SMITH, 1927, p. 485.

Specimens Examined—S.M. A. 6270-2, A. 7207-18, O. A. JONES Collection, from the Lower Visby Marls, shore north of Visby, Gotland; R. 25555-62, G. J. HINDE Collection, B.M., Silurian, Gotland; R30517-9, B.M., Silurian, Gotland; S.M. A7024-5, D. HILL Collection, Upper Llandovery, Malvern tunnel tip-heap, Colwall Station, Malverns; S.M. A7026-34, D. HILL Collection, Lower Wenlock, Marloes Bay, Pembrokeshire.

General Description—The corallum is simple, small, and discoid, and has an average diameter of 12 mm. From a minute, centrally-placed, and erect cone of attachment, the epitheca expands rapidly into a flat disc (fig. 26). It shows fine annulations,

and rarely, interseptal ridges. The acanthine septa, about 22 of each order, are borne on the upper surface of the epitheca, and show the four points of septal insertion of the Rugosa. The minor septa are about two-thirds as long as the major. There are no tabulae or dissepiments.

Septal Structure—The free edge of the septum is arched, its highest point being about half-way between the periphery and the axis of the corallum (fig. 14C). Viewed from above (fig. 26C) it shows a single series of low projections. The septum appears slightly constricted between the projections, which in transverse section are irregularly rounded, stellate, or transversely elongate. In the upper parts of the corallum the septa are free laterally, but in the lower parts they are dilated and in contact.



FIGS. 8-10—Types of Trabeculae

FIG. 8—Monacanth. A, in median vertical section; B, in transverse section.

FIG. 9—Rhabdacanth. A, in median vertical section; B, in tangential vertical section; C, in transverse section; D, E, a "rod" as it appears in median vertical and transverse sections; F, G, the hypothetical structure of a rod, in median vertical and transverse section.

FIG. 10—Holacanth. A, B, in median vertical and transverse section.

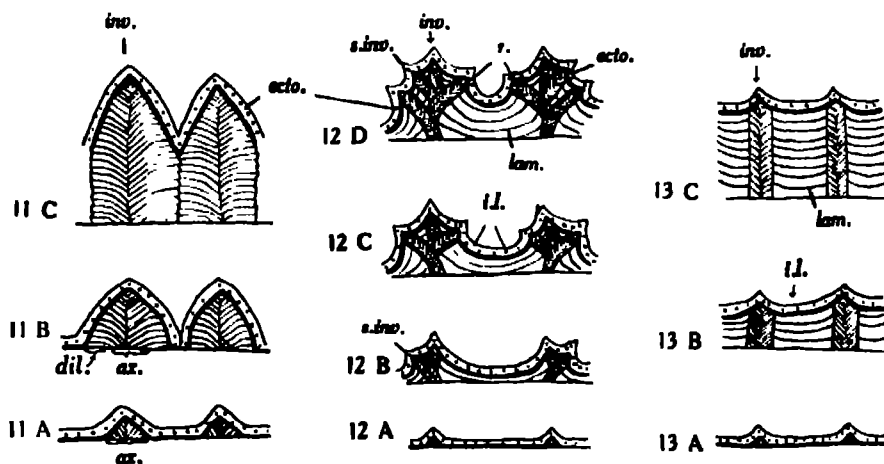
Fib., fibres; *lam.*, lamellar sclerenchyme; *r.*, "rod".

The microscope shows that each septum consists of fibres (fig. 14, and fig. 36, Plate 29), which are grouped about a number of axes in the median plane. The axes are directed obliquely upwards and outwards from the epitheca, and the fibres around each compose a spiniform trabecula. Each trabecula consists of an undilated axial part, whose end forms a projection from the free edge of the septum, and dilated lateral parts by which neighbouring septa are placed in contact. In the axial parts of the trabecula the fibres are directed radially upwards, but laterally, *i.e.*, where they face neighbouring septa, they change their direction and come to lie at 90°, or even as much as 120°, to the axis. Such a trabecula is here called a *monacanth* (figs. 8A-B).

Each radial series of monacanth forms a continuous plate which is here called a *monacanthine septum* (fig. 14). An irregular original suture is always present between the dilated parts of neighbouring septa.

Variability—The monacanthi vary in inclination from almost erect to almost horizontal, and in some specimens the obliquity is very pronounced. In a transverse section of the corallum the axis of an almost erect monacanth appears as an irregularly rounded spot, but that of a more oblique trabecula appears as a line (fig. 14B). The shape of the trabeculae projecting from the free edge of the septum also is variable; one of the variations is reminiscent of the elongated yard-arm carina of *Heliophyllum* Hall.

Morphology and Histology—The fibrous structure of the septum is best observed with a 1-inch objective and crossed nicols. Recrystallization invariably prevents



FIGS. 11-13—Formation of Trabeculae. Each drawing represents a median vertical section through the trabeculae of two neighbouring septa, and the tissue between.

FIG. 11—A, B, C, three stages in the formation of monacanthi.

FIG. 12—A, B, C, D, four stages in the formation of rhabdacanths.

FIG. 13—A, B, C, three stages in the formation of holacanths.

Ax., fibres secreted by the top of the invagination; dil., fibres secreted by the sides of the invagination; ecto., ectoderm; inv., invagination in the base of the polyp; lam., lamellar sclerenchyme laid down by unfolded parts (i.l.) of basal ectoderm; r., "rods" of the rhabdacanth; s.inv., subsidiary invagination where formation of rod is completed.

measurement of the individual fibres, and I have been unable to determine whether, or not, they are grouped in the conical fascicles demonstrated by OGILVIE in the trabeculae of Recent Corals (1897, p. 117). Frequently there is a suggestion that the trabecula includes a number of rod-like aggregates of fibres, with a light axis and a dark margin, similar to those described later in *Acanthocyclus* and *Tryplasma*.

The following explanation of the formation of the septa of *P. porpita* seems reasonable. Secretion of a septum was begun in a radial series of small, isolated, conical invaginations* in the base of the polyp. From each a monacanth was

* This is in agreement with the facts observed by VON KOCH (1882, a) in *Asteroides calycularis* and by DUERDEN (1904) in *Siderastraea radians*.

eventually formed. As the individual grew, the invaginations grew taller, and their lower parts widened till they coalesced to form a deep radial groove with conical invaginations along its summit (fig. 1). New growths of fibres were continually begun at the apices, and added to by the sides, as the invaginations increased (fig. 11). The fibres were always laid down at right angles to the secreting surface, so that those formed at the apex were directed upwards, and those added from the coalesced sides were horizontal and constitute the lateral or "dilated" part of the monacanth. Presumably new cells (or special parts of the ectodermal syncytium, if we follow BOURNE) began to secrete the fibrous deposit at the apex, and their division-products added layers to the fibrous deposit as they were left deeper and deeper in the apically-growing invagination. Deposition of fresh layers of fibres continued, until the dilated parts of neighbouring septa were in contact, and the fold of soft tissue between each invagination was forced to atrophy or to withdraw, by being crushed between the two neighbouring fibrous aggregates. There is no evidence that the unfolded parts of the ectoderm of the base, lying between two neighbouring invaginations, ever laid down any sclerenchyme.

Phylogeny—This species is regarded as the ancestor of *Acanthocyclus porpitoides*. The possibility is discussed after describing the septal structure of the latter.

Palaeocyclus rugosus EDWARDS and HAIME

Palaeocyclus rugosus EDWARDS and HAIME, 1851, p. 206; 1854, p. 248, Pl. lvii, figs. 4, 4a-d, from the Silurian of Dudley and Wenlock.

Two of the specimens figured by EDWARDS and HAIME (those figured, 1854, Plate lvii, figs. 4a, 4c, S.M. A6582, A6584) have been sectioned, and found to be congeneric with neither *Palaeocyclus porpita* (LINNAEUS) nor *Acanthocyclus fletcheri* (EDWARDS and HAIME). Their septa are not acanthine; they have a columella, and are probably related to "*Cyathaxonia*" *dalmani* EDWARDS and HAIME. The two specimens, figs. 4, 4b (S.M. A6581, A6583), have not been cut, but I have no doubt that they are conspecific with those represented in figs. 4a, 4c.

Genus *Acanthocyclus* DYBOWSKI

Acanthocyclus DYBOWSKI, 1873, p. 103 (359).

Acanthocyclus DYBOWSKI; LANG and SMITH, 1927, p. 450.

Genolectotype, *Palaeocyclus fletcheri* EDWARDS and HAIME, 1851, p. 205; 1854, p. 248 and Plate lvii, figs. 3, 3a-f. Silurian, Dudley. Chosen LANG and SMITH, 1927, p. 450.

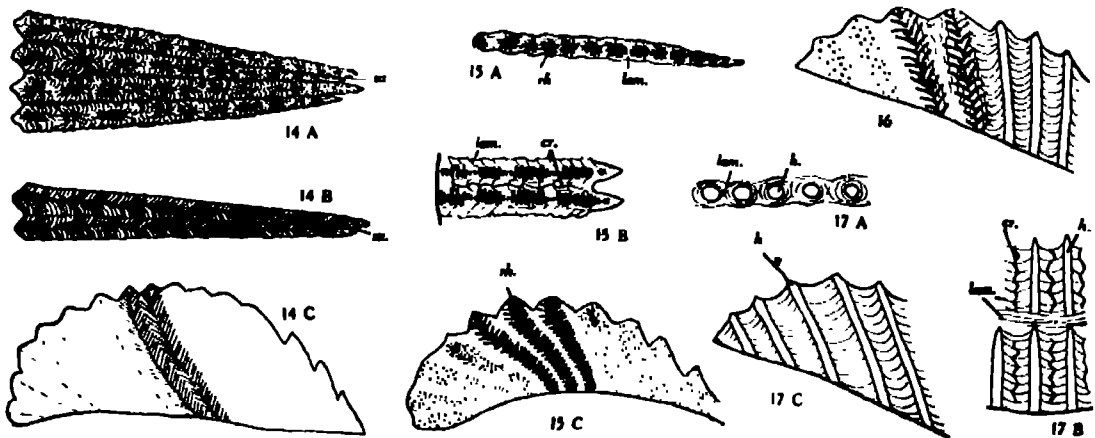
Acanthocyclus porpitoides LANG and SMITH. Figs. 9, 12, 15, 19, and 27, and Fig. 37, Plate 29.

Acanthocyclus porpitoides LANG and SMITH, 1927, p. 486.

Specimens examined.—S.M. A5927-32, 6286, 6433-4, 6846-7 (which are the specimens figured by EDWARDS and HAIME, 1854, Plate lvii, figs. 1, 1a, 1b, 1c, as

Palaeocyclus porpita LINNAEUS), 6848, 7204-6, 7220-7, 7448, from Dudley. Mr. A. J. BUTLER, who has recently made a detailed investigation of the stratigraphy and coral fauna of Dudley, thinks it probable that most of the specimens of *Acanthocyclus* collected from Dudley were found in the Wenlock Shales below the limestones, at the time when a tunnel was cut through Wren's Nest.

General Description—The corallum is simple, small and discoid, and has an average diameter of 14 mm. The epitheca expands rapidly from a minute, excentric, and obliquely directed cone of attachment, to an almost flat disc (fig. 27). It shows growth-wrinkles, and, rarely, septal sulci. The acanthine septa, about 30 of each



FIGS. 14-17—Types of Septa.

FIG. 14—Monacanthine. A, four septa in transverse section cut at right angles to the trabeculae ; B, two septa in transverse section cut obliquely to the trabeculae ; C, one septum in vertical section, the structure of only two trabeculae being shown.

FIG. 15—Rhabdacanthine. A, in transverse section cut at right angles to the trabeculae ; B, in transverse section cut obliquely to the trabeculae ; C, in vertical section, the three central trabeculae being shown in median vertical section, and the rest in tangential vertical section.

FIG. 16—Diniorphacanthine. In vertical section ; the two rhabdacanths at the left are in tangential vertical section ; the next two are in median vertical section ; the three trabeculae at the right are holacanths.

FIG. 17—Holacanthine. A, in transverse section cut at right angles to the trabeculae ; B, in transverse section cut obliquely to the trabeculae ; C, in vertical section.

cr., cracks ; h., holacanth ; lam., lamellar sclerenchyme ; rh., rhabdacanths ; su., original suture between two monacanthine septa.

order, are borne on the upper surface of the epitheca, and show the four points of insertion of the Rugosa. The minor septa are two-thirds as long as the major. There are no dissepiments nor tabulae.

Septal Structure—The free edge of the septum is arched, its highest point being about half-way between the periphery and the axis of the corallum (fig. 15C). It has a number of projections, usually in single series, but there may be two series near the periphery, placed alternatively or in pairs (fig. 27C). Viewed from above, the projections usually appear stellate.

When examined with a microscope, each septum appears to consist of two distinct types of sclerenchyme (fig. 15, and fig. 37, Plate 29). The first type forms spines, whose tops are the projections from the free edge. The second type consists of lamellae, which bind the spines into a compact plate. The spines have grown in the plane of the septum upwards and outwards from the epitheca, and have an average diameter of 0.5 mm. Each is entirely made up of "rods", directed upwards and outwards from the axis. The "rods" are necessarily more crowded at the axis of the spine than at the periphery, but no separately-formed axial pillar can be distinguished. Such spines are here called *rhabdacanth*s (fig. 9). In some the "rods" are widely spaced and very long, and their projections make the spine appear prickly; in others the "rods" are closely spaced and shorter, so that the rhabdacanth appears coarsely fibrous and end in smooth projections. The outline of the "rod" is dark and hazy, and circular in transverse section; the average diameter is 0.05 mm. The axis is transparent. Recrystallization usually masks the ultimate structure, but sometimes, when a transverse section of a rod is examined under crossed nicols, the suggestion of a cross is seen, indicating that the rod consists of fibres directed radially from its axis. A rhabdanth is probably a compound trabecula, as each rod seems to have the structure of a very small, simple trabecula.

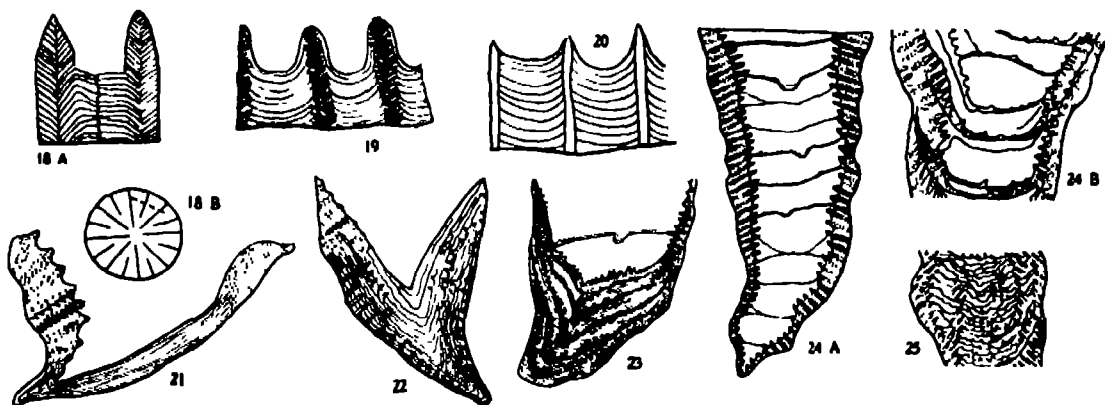
Binding each series, or double series, of rhabdacanth into a compact septum, and each septum to its neighbour, is the second type of sclerenchyme. It consists of a large number of excessively thin, parallel lamellae (fig. 19). In the interseptal loculi between the bases of the septa, the lamellae are parallel to the epitheca, but they arch up along, and abut against, the sides of each septal series of rhabdacanth (fig. 9). In rare cases some of the rhabdacanth are swathed separately. Proof of the lamellar nature of this sclerenchyme is obtained by cutting sections parallel to the lamellae and observing that the divisional planes disappear, or are seen as a series of contour lines around some sectioned eminence. The difference between this lamellar sclerenchyme and the fibrous dilating sclerenchyme of *Palaeocyclus* is quite obvious in thin section (compare fig. 36, Plate 29, with fig. 37, Plate 29). Owing to recrystallization it is impossible to prove whether each lamella was laid down as a single crystal, or as an aggregate of very short fibres, each with its long axis at right angles to the plane of the lamella; but appearances under crossed nicols suggest the latter condition.

Such septa, which consist of contiguous rhabdacanth bound into a plate by lamellar sclerenchyme, are here called *rhabdanthine septa*.

Variability—The relations of the lamellae and the rhabdacanth are variable. Usually the septal series is swathed as a whole by the lamellae, but occasionally a rhabdanth is separately enclosed. A septum may sometimes contain two series of rhabdacanth, either alternating, or placed side by side. This shows that compound septa are possible in *Rugosa*, and explains many puzzling facts in other genera.

Morphology and Histology—The nature of the "rods": The occurrence of "rods" in individuals of this species is constant enough to be a diagnostic character. Elsewhere they are known only in other species of *Acanthocyclus*, in the closely related

genus *Tryplasma*, and in *Cantrillia*, and they are characteristic of such species whatever the horizon or locality. These facts, with the constancy of their shape and size and positional relation to the spines, show that they are either appearances dependent on the original structure of the spine, or original parts of it. It is conceivable that the appearance of rods in transverse and vertical sections of a spine could be obtained without the actual presence of rod-like bodies, provided that the spine consisted of fibres directed radially upwards. Such appearances are sometimes observed in the septa of Recent Corals, when dark rod-like areas follow the grain of the fibres, and



FIGS. 18-25—Types of Horizontal Tissue

- FIG. 18—A, *Palaeocyclus porpita*; has no horizontal tissue; diagram shows neighbouring septa in contact by means of dilatation of their fibrous tissue. B, Diagram of calice with broken line showing direction taken by vertical sections of figs. 18A, 19, and 20.
- FIG. 19—*Acanthocyclus porpitoides*; diagram showing relation of horizontal tissue (lamellar sclerenchyme) to rhabdacanthine septa.
- FIG. 20—*Tryplasma primum*; diagram showing relation of horizontal tissue (lamellar sclerenchyme) to holacanthine septa.
- FIG. 21—*Acanthocyclus fletcheri*; lamellar sclerenchyme.
- FIG. 22—*Acanthocyclus transiens*; cross-bedded lamellar sclerenchyme.
- FIG. 23—*Tryplasma primum*; lamellar sclerenchyme and notched tabulae.
- FIG. 24—*Tryplasma loveni*; A, with some notched tabulae; B, with tabulae whose formation may be connected with rejuvenescence.
- FIG. 25—*Tryplasma malvernense*; close, concave tabulae, peripherally following discontinuities in the septa.

are doubtless due to some infilling of the spaces or alteration of the fibres. That this is not so in *A. porpitoides* is proved by the tangential section of a rhabdacanth (fig. 9B; see also fig. 38, Plate 29); for here the "rods" have a most characteristic and constant size and form, and a definite circular outline, such as could not be expected from fortuitous alteration patches in a fibrous aggregate. Furthermore, each rod causes the lamellar sclerenchyme to arch up about it. Consequently, we must accept the postulate that these rod-like bodies are entities which have taken part as such in the construction of the spine. The "rods" are the unit of structure

for the spine, and therefore for the septum of *Acanthocyclus*. From their typical appearance under the microscope, it is conceivable that the "rods" may be tubes, spicules, bundles of fibres, or rods with a granular structure. It would not be in accordance with our knowledge of the corallum for the "rods" to be tubes; if they are spicules, EDWARDS's and HAIME's views of the unit of coral structure would be supported in part, but this also seems unlikely. If they are bundles of fibres, there is no conflict at all with current theory. While they may be of granular matter, I consider it almost certain that each is a very small trabecula, and consists of fibres directed radially from its axis, since sometimes in transverse sections of the less recrystallized rods, a faint cross is shown under crossed nicols.

The Nature of the Lamellae—As stated above, recrystallization has made it impossible to prove whether each lamella was originally a single plate-like crystal, or a sheet of fibres arranged with their long axes at right angles to the surface of the lamellae; but appearances under crossed nicols suggest that the latter is the case, and this is in accordance with current ideas on the coral skeleton. OGILVIE shows that in certain Hexacorals (*Goniastrea*, 1897, p. 148) the arrangement of the calcareous fibres in growth-lamellae is more obvious than the fibrous nature of the lamellae. I consider that such an arrangement is characteristic of the Silurian genera *Acanthocyclus*, *Tryplasma*, *Cystiphyllum*, and *Cantrillia*, and in order to emphasize the predominance of the growth lamellae over the normal fibrosity, I shall speak of the *lamellar dilating sclerenchyme* of these genera.

The Formation of the Septa (fig. 12)—The general process of septum-formation was similar to that already described for *Palaeocyclus*. But, instead of only the conical invaginations, the fold of ectoderm in each interseptal loculus also had the power to secrete sclerenchyme. Further, in each conical invagination (in which each rhabdacanth was secreted) there were subsidiary pointed invaginations, in each of which a "rod" was secreted. Each "rod" was begun at the apex of the conical invagination, and added to by its parent cells (or special parts of the ectodermal syncytium, if we follow BOURNE) or their division-products. These remained behind and formed a subsidiary pointed invagination when new cells (or special parts of the syncytium) were inserted at the apex to secrete the beginnings of a new rod. Cells (or parts of the syncytium) arising between the subsidiary invaginations during the growth of a conical invagination had a different secretory function. Like the cells or parts of the fold of ectoderm in an interseptal loculus, they secreted lamellar sclerenchyme. The lamellae connected neighbouring septa, and at first sheathed the rhabdacanths separately, but later, when neighbouring rhabdacanths coalesced, united them in septal series.

Phylogeny—*A. porpitoides* is thought to have evolved from *P. porpita*. Their external similarity suggests that they are closely related. They are both discoid forms with acanthine septa whose trabeculae are of the same size, but *A. porpitoides* has more septa, and presumably a less primitive corallum. The rhabdacanth of *A. porpitoides* might well be considered a specialization from the monacanth of *P. porpita*, since it entails the development of subsidiary invaginations; the occurrence of horizontal

tissue, accompanied by, and perhaps due to, the dominantly lamellar condition of the dilating sclerenchyme, is an advance on *P. porpita*, where no horizontal tissue is differentiated. Further, these very changes are the ones which, by further evolution, gave rise to a series of species of *Acanthocyclus* leading to *Tryplasma*. Stratigraphical evidence is in accordance with this assumption of descent. Thus, in most localities, *P. porpita* is confined to the Upper Llandovery, while *A. porpitoidea* is a Wenlock Shale form. In Marloes Bay, *P. porpita* is found with a Wenlock Shale fauna.

Acanthocyclus fletcheri (EDWARDS and HAIME). (Figs. 21 and 28)

Palaeocyclus fletcheri EDWARDS and HAIME, 1851, p. 205 ; 1854, p. 248, Plate lvii, figs. 3, 3a-f ; Silurian ; Dudley.

Acanthocyclus fletcheri (EDWARDS and HAIME) ; LANG and SMITH, 1927, p. 450.

Lectotype (here chosen) : S.M. A6850, specimen figured EDWARDS and HAIME, 1854, fig. 3d ; here figured as fig. 28.

Specimens Examined—S.M. A5925, 5939-40, 6849-50, 7085-93, 7229-33, 7276-7, 7444-5 ; Dudley ; for horizon, see remarks under *A. porpitoidea*. S.M. A7035-37, Wenlock Shale of Malvern Tunnel tip-heap, Colwall Station, Malverns.

General Description—The corallum is simple, curved, and patellate, with an average diameter of 15 mm, and a calice so deeply excavated that its floor is parallel to the epitheca (fig. 21). The epitheca shows fine annulations, and, rarely, interseptal ridges arranged in double-ribs with epithecal scales. The acanthine septa, about 38 of each order, show the four points of septal insertion of the Rugosa. There are no dissepiments or tabulae. Rejuvenescences sometimes occur, and, rarely, peripheral offsets.

Septal Structure (fig. 16)—The free edge of the septum is formed by a number of tall disconnected projections, a single series to each septum. The microscope shows that the septum is very similar to that of *A. porpitoidea*, but the rhabdacanthi are further apart and are more frequently embedded separately in lamellar sclerenchyme. Also, in the deeper parts of the corallum of *A. fletcheri* smooth spines may be seen, which show no trace of "rods", are about half the diameter of the rhabdacanthi, and tend to be placed more closely together. Such trabeculae are here called *holacanthi* (fig. 10). In all specimens examined, recrystallization masks their structure. The lamellae arch up and about the rhabdacanthi and holacanthi, and appear to pass into them. The trabeculae are perpendicular to the main plane of the lamellae in this and all other species where such lamellae are developed. Septa like these (fig. 16), which consist of a vertical series of rhabdacanthi and holacanthi embedded in lamellar sclerenchyme, are here called *dimorphacanthine septa*.

Variability—This species is very variable. It normally exhibits a curved patellate corallum, in which the horizontal tissue is thin. Specimens S.M. A7274-5 ; 7278-81, and 7446-7, from Dudley, are turbinate, or even trochoid, and the horizontal tissue is much thicker than in typical specimens. These individuals are transitional to

A. transiens, described below. Some specimens are erect, with the cone of attachment in the centre of the base, and some of these are tall and trochoid. A7296 is an erect trochoid form, in which tabulae are differentiated from the thickened horizontal tissue. It thus anticipates the further evolution of the group towards *Tryplasma*. One or two rejuvenescence-rims are seen in many coralla, and three show peripheral offsets ("buds"). Rejuvenescence and peripheral increase are typical of the species evolved from this stock in Wenlock Limestone times. The number of the septa is not constant. Two types of trabeculae occur, and the distance between trabeculae is variable.

Morphology and Histology—The general process of septum-formation was similar to that of *Acanthocyclus porpitoides*. But the conical invaginations, which normally secreted rhabdacanth, alternately could secrete holacanth. For the formation of a holacanth (fig. 13) it is supposed that only the apex of the invagination secreted the trabecula. As new apical cells (or special parts of the ectodermal syncytium, if we follow BOURNE) were introduced, the old apical cells (or parts of the syncytium) failed to remain as subsidiary invaginations, and took on the function of secreting lamellar sclerenchyme. They laid down material in continuity with the lamellae formed by the fold of ectoderm in each interseptal locus.

Phylogeny—*A. fletcheri* was almost certainly derived from *A. porpitoides*, which it closely resembles, by a change of shape from discoid to patellate, an increase in the number of septa, a change in the structure of the trabeculae, and a stronger development of lamellar sclerenchyme. By an extension of the same changes it leads on to *Acanthocyclus transiens* sp. nov., and thence to *Tryplasma primum* sp. nov.

Acanthocyclus transiens sp. nov. (Figs. 16, 22, and 30, fig. 40, Plate 29, and figs. 41 and 42, Plate 30)

Compare with *Cyathophyllum pileolum* QUENSTEDT, 1881, p. 455, Plate 158, fig. 23, Silurian, Gotland.*

Holotype—S.M. A6851, Wenlock Shale, Malvern tunnel tip-heap, Colwall Station, Malverns (fig. 30 ; fig. 41, Plate 00).

Specimens Examined—S.M. A6435-7, A 7314-9, from the type locality ; one from the Wenlock Shale, Titford Shaft (400 yards down), South Staffordshire Coalfield ; A5936-8, 7124-7, 7254, 7294-5 and 7305 from Dudley.

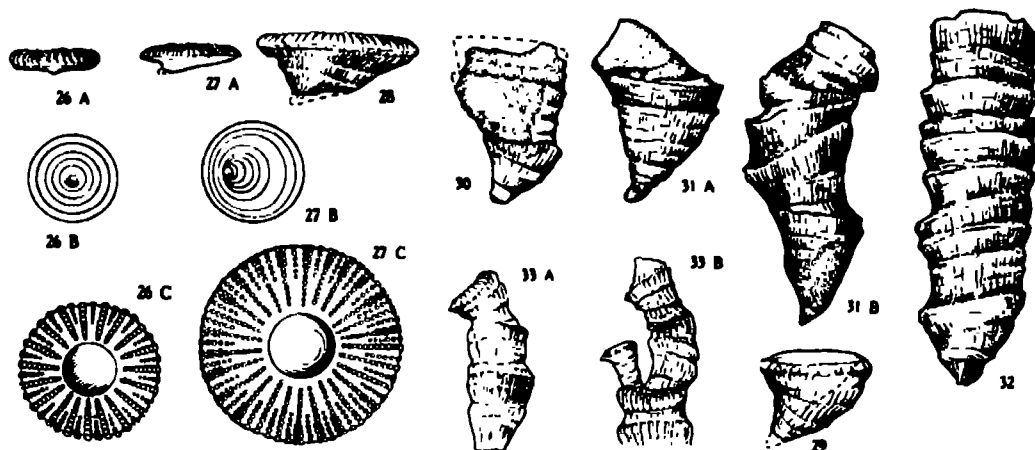
Diagnosis—Turbinat *Acanthocyclus* with a calice half as deep as the corallum.

General Description—The corallum is simple, curved, and turbinat or trochoid, and the calice extends half-way to the apex (fig. 30). The coralla average 15 mm in both diameter and height, and some show one or two rejuvenescence rims. The epitheca shows double-ribs. The septa are acanthine and crowded, the minor septa (28-32) being half, or more than half, as long as the major septa with which

* QUENSTEDT's specimen is not at present accessible to me ; the English specimens resemble it very closely in external characters.

they alternate. Each spine is separately embedded in lamellar sclerenchyme, which fills the corallum from tip to calice (fig. 41, Plate 30). Tabulae and dissepiments are absent.

Septal Structure—The septa are dimorphacanthine (fig. 16), and the number of holacanth is frequently greater than the number of rhabdacanth. Most of the trabeculae are separately embedded in the lamellar sclerenchyme, which fills the rest of the lumen and usually shows cross-bedding



FIGS. 26-33—External Characters.

- FIG. 26—*Palaeocyclus porpita*; discoid corallum; A, side view; B, basal view, cone of attachment central; C, calical view; septa of uniserial spines.
 FIG. 27—*Acanthocyclus porpitoides*; discoid corallum; A, side view; B, basal view, cone of attachment excentric; C, calical view; septa often of two series of spines.
 FIG. 28—*Acanthocyclus fletcheri*; patellate corallum.
 FIG. 29—*Acanthocyclus* aff. *transiens*; broadly trochoid corallum.
 FIG. 30—*Acanthocyclus transiens*; broadly trochoid corallum.
 FIG. 31—*Trylasma primum*; trochoid corallum. A, showing rejuvenescence entailing gradual reduction of diameter; B, showing cone-in-cone rejuvenescence.
 FIG. 32—*Tryplasma loveni*; corallum trochoid at first, showing repeated cone-in-cone rejuvenescence.
 FIG. 33—*Tryplasma malvernense*; ceratoid corallum. A, with cone-in-cone rejuvenescence; B, with peripheral offsets ("buds").

FIGS. 26C and 27C, $\times 2$ diameters; remainder natural size.

Variability—Some individuals are variants towards *A. fletcheri* in shape, septal structure, and development of lamellar sclerenchyme; others approach *Tryplasma primum* sp. nov.

Morphology and Histology—Septum-formation was similar to that in *A. fletcheri*. Cross-bedding of the lamellar sclerenchyme (fig. 22, and fig. 40, Plate 29) results when groups of trabeculae grow more quickly than the remainder, causing related parts of the base of the polyp to move further in a vertical plane than the parts unaffected by the stresses set up. The location for the most rapidly moving segment changes occasionally, so that the lamellae come to lie at different angles.

Phylogeny—It is clear that this species has been derived from *A. fletcheri*, since it occurs at the same localities, has the same number of septa, and is connected with it by a continuous series of forms. It leads on to *Tryplasma primum*. The changes involved were (1) a shape-change in the curved coralla, from patellate to turbinate and trochoid forms ; (2) an increase in the proportion of holacanth to rhabdacanth, and in the number of separately embedded trabeculae ; (3) an increase in the amount of lamellar sclerenchyme ; it becomes apparent that this has the function of horizontal tissue.

Acanthocyclus aff. *transiens* (Fig. 29 ; fig. 38, Plate 29)

It is proposed thus to refer to 26 coralla (A6439-41 ; 7320-30 ; 7393-7414) from the Lower Ludlow Shales of Ledbury Quarry, collected by Miss F. E. S. CALDWELL. Their septa vary between 24 and 34 of each order. In most of their characters, these forms are less advanced than *A. transiens*. Thus they have a more patellate shape, a less thick deposit of lamellar sclerenchyme, and the trabeculae are mostly rhabdacanth. But they are more advanced than *A. transiens* in that many of them (S.M. A6441, 7321, 7330, 7402, 7404-6, 7408) develop one or two tabulae just below the calice, so that, like *A. fletcheri* and *A. transiens*, they lead on to *Tryplasma*. One individual, A7408, has a peripheral offset. The proto-corallite is *Acanthocycloid* but the bud is *Tryplasmoid*. They thus form an excellent example of orthogenesis with the *Acanthocyclus-Tryplasma* lineage of the Wenlock Shales.

Acanthocyclus binus (LONSDALE)

Turbinolopsis bina sp. nov. LONSDALE, 1839, p. 692, and Plate xvi bis, figs. 5, 5a.

Acanthocyclus binus (LONSDALE) ; LANG and SMITH, 1927, p. 483, Plate xxxvii, fig. 5 ; Upper Llandovery ; Marloes Bay, Pembrokeshire.

This form is much smaller and more slenderly conical than *A. transiens*. I have not examined any specimens ; the Marloes Bay corals are too recrystallized for detailed study in septal structures. The mould of the holotype suggests a ceratoid *Acanthocyclus* with the internal structure of *A. transiens*.

Genus *Tryplasma* LONSDALE

Tryplasma LONSDALE, 1845, p. 613.

Tryplasma LONSDALE ; LANG and SMITH, 1927, p. 461.

Tryplasma primum sp. nov. (Figs. 16, 17, 20, 23 and 31 ; fig. 39, Plate 29 ; figs. 43-45, Plate 30.)

Holotype—S.M. A6445 ; Wenlock Shale ; Malvern Tunnel tip-heap, Malverns (fig. 43, Plate 30.)

Specimens Examined—S.M. A6442-7, 6853, 7197-7200, 7355-69 from the type locality ; 5706-7, 7098-9, 7245, from Dudley (for horizon see under *A. porpitoideus*) ;

A5695, from Walsall. A6854, A7370-1, from the Wenlock Shale of Titford Shaft (400 yards down), South Staffordshire Coalfield. A7430-5, Salopian; Barber's Quarry, Falfield, Tortworth inlier, Gloucestershire.

Diagnosis—Simple, trochoid *Tryplasma* with occasional rejuvenescence, dimorph-acanthine septa, and a few thick tabulae formed of lamellar sclerenchyme.

General Description—The corallum is simple, curved, and trochoid. The average height is 25 mm, and the average adult diameter 15 mm. Rejuvenescence is of two types, and occurs once to three times in a corallum. In the less frequent type the diameter gradually decreases from a pronounced growth-swelling (fig. 31A); in the other, a new corallite grows up, fitting inside the old calice—*cone-in-cone* type (fig. 31B). The epitheca is weakly double-ribbed. There are about 40 long acanthine septa of each order, very closely spaced, the minor septa usually showing little difference in length from the major. The proximal parts of the corallum are filled with lamellar sclerenchyme; but in the distal, less curved parts, and frequently coinciding with a rejuvenescence ridge, are a few flat, widely spaced tabulae, formed of lamellar sclerenchyme, and usually very thick. The tabulae may have an axial concave notch (fig. 23). There are no dissepiments. The tissue of the corallites has a yellowish tinge.

Septal Structure—The septa are dimorphacanthine, as in *Acanthocyclus transiens*; each trabecula is separately embedded in lamellar sclerenchyme (figs. 16-17; figs. 43-45, Plate 30). The axial edges of the minor septa are covered by late deposits of this sclerenchyme, which are laid down in the loculi between the axial edges of neighbouring major septa. Each tabula may consist of one lamella stretching completely across the tabularium; or numbers of such complete lamellae; or an echeloned series of partial lamellae (fig. 39, Plate 29).

Variability—Two types of rejuvenescence are possible, as described above. The height of the corallum is variable; some individuals immediately transitional from *A. transiens* have only one tabula; taller coralla may have many. There is wide variation in the height at which the first tabula is developed.

Morphology and Histology—The great interest of this species is the occurrence of tabulae (fig. 23; figs. 43-45, Plate 30). In *Acanthocyclus*, as we have seen, those parts which in other groups lay down the horizontal tissue in the form of tabulae and dissepiments, take on (as do the sides of the septal invaginations) the function of laying down lamellar sclerenchyme. In *Tryplasma*, however, we have the comparatively sudden appearance of tabulae, but not of dissepiments, although the minor septa are very long. Considering the length of the minor septa, the absence of dissepiments is even more surprising than the occurrence of tabulae, because in other groups of the Rugosa the development of the minor septa is correlated with that of the dissepiments. After the base of the polyp has risen slowly by the deposition behind it of lamella after lamella, its axial parts are suddenly uplifted through a distance corresponding to the thickness of (possibly) a hundred lamellae, so that a space is left, which is bounded towards the periphery by the lamellae at the axial ends of the minor septa. That this space is due to a sudden upward

movement of the axial part of the base of the polyp, and not to a temporary cessation of the secreting powers of this part of the ectoderm, is proved by the fact that the peripheral extensions of the lamellae laid down to form the tabula follow immediately the peripheral extensions of the lamellae at the bottom of the space (fig. 43, Plate 30).^{*} The development of tabulae, or rather of inter-tabulate spaces, is due to the periodic relief of stress in the base of the polyp (*see* Introduction). Stress arises when the peripheral parts of the corallum grow vertically more quickly than the axial parts. This differential growth results when the rate of deposition of fibres is greater at the apices of the trabecular invaginations than along the uninvaginated parts of the base. It is also probably assisted in some way by the phylogenetic assumption of the trochoid form of the corallum. In some cases tabula-formation may be connected with rejuvenescence.

Phylogeny—This species was derived from *A. transiens*, with which it occurs, by (1) a change in shape from turbinate to trochoid; (2) an increase in the power of rejuvenescence; (3) an increase in the number of trabeculae separately embedded in lamellar sclerenchyme; (4) the formation of inter-tabulate spaces by the development of tabulae. By an extension of these changes it leads on to *Tryplasma loveni* (EDWARDS and HAIME).

Tryplasma loveni (EDWARDS and HAIME). (Figs. 24 and 32; figs. 46 and 47, Plate 30)

Cyathophyllum (?) *Loveni* EDWARDS and HAIME, 1851, p. 364.

Cyathophyllum (?) *Loveni* EDWARDS and HAIME, 1854, p. 280, Plate lxvi, fig. 2.

Type Material—When I visited the Natural History Museum of Paris, I was informed that the figured specimens from Wren's Nest, Dudley, were not there. Nor did I find them in the School of Mines, Paris. It is possible that they are in that part of the collection of M. BOUCHARD-CHANTEREUX believed to be in Boulogne. Pending their rediscovery, I interpret the species by topotypes, S.M. A8166-7, from Wren's Nest, Dudley (from the top 10 ft of nodular beds below the Upper Limestone), and A7100-1, 7349-50, in the Fletcher Collection from Dudley. These agree with EDWARDS's and HAIME's fig. 2, but not with 2a, which shows dissepiments and probably represents another genus.

Specimens Examined—The topotypes mentioned above; S.M. A6413, 6415, 6448, 6855, 7387-92, from the Wenlock Limestone of Wenlock Edge.

Diagnosis—Simple trochoid *Tryplasma*, with repeated cone-in-cone rejuvenescence and rhabdacanthine septa.

General Description—The corallum is curved, and trochoid at the apex, but typically erect, with repeated cone-in-cone rejuvenescence distally (fig. 32). Less typically rejuvenescence of a second type occurs, giving upright cones alternating with the

^{*} BUTLER (1935, p. 123) has already made this observation, in describing the tabulae of *Syringaxon siluriensis* (M'COY).

normal inverted cones. The average diameter attained is about 15 mm, and the average height about 40 mm. Two specimens show peripheral offsets. The epitheca usually shows marked double-ribs. Some specimens show scales on the epitheca. The average number of septa is 36 of each order; the major septa are one-third, to half, as long as the radius of the corallum, and the minor septa are half as long as the major septa. Each septum consists of a vertical series of spines, embedded in a peripheral stereozone of lamellar sclerenchyme almost as wide as the length of the major septa. The tabulae are distant, usually unthickened, horizontal, or slightly concave, often with an axial notch (fig. 24A; fig. 47, Plate 30). They frequently correspond to rejuvenescence-ridges higher in the corallum (fig. 24B); sometimes they continue sharply upwards to the epitheca, and there is a space below them in the stereozone. There are no dissepiments.

Septal Structure—The trabeculae are given off from the epitheca at about 45°, but towards their axial ends they may approach the horizontal. They are rhabdacanthi (fig. 24; fig. 47, Plate 30), and the septa are typically rhabdacanthine.

Variability—The species varies within fairly wide limits. Much of this variability is connected with rejuvenescence. Thus, one type of rejuvenescence gives coralla consisting of inverted trochoid segments alternating with trochoid segments, while most coralla show repeated cone-in-cone rejuvenescence, each segment being trochoid. In most coralla the tabulae are flat, and cannot be traced in the stereozone, but in some they are bucket-shaped, for they are continued sharply upwards through the stereozone to the epitheca, following a discontinuity in the stereozone. Some coralla have fewer and shorter septa, tabulae close together, and a smaller diameter. S.M. A7245, 7289–90 are transitional from *T. primum*.

Morphology and Histology—In some coralla the tabulae are continuous upwards to the epitheca, following a discontinuity in the stereozone. It seems likely that this particular condition is due to rejuvenescence, for in the rare instances where such coralla show external rejuvenescence-ridges, the periphery of the tabula coincides with the epitheca of the new segment (fig. 24B). The evidence from *Tryplasma loveni* suggests that two reasons for tabula-formation are possible, the greater deposition of fibres at the apices of trabecular invaginations (as explained under *T. primum*), and rejuvenescence. BERNARD has already suggested a connexion between tabula-formation and rejuvenescence, which he explained as transverse fission (BERNARD, 1906, p. 23; LANG, 1909, p. 292).

Phylogeny—This group is almost certainly derived from *Tryplasma primum*, with which it is connected by transients, just as *T. primum* was derived from *Acanthocyclus transiens*, by a further evolution affecting shape, rejuvenescence, and horizontal tissue; but the trend in the degeneration of the septa has been slightly reversed, and *T. loveni* shows rhabdacanthine septa like *A. fletcheri*, the ancestor of *A. transiens*. The species shows that recapitulation is very quickly concluded, for while *T. primum* still has most of its apex filled with lamellar sclerenchyme, showing its origin from *A. transiens*, its descendent *A. loveni* has no more lamellar sclerenchyme developed at its apex than on its sides.

Tryplasma malvernense sp. nov. (Figs. 25 and 33 ; figs. 48 and 49, Plate 30)

Compare with *Tryplasma articulatum* LONSDALE, 1845, pp. 613, 633, Plate A, figs. 8, 8a-e ; *non* WAHLENBERG (whose material was collected in Gotland).

Holotype—S.M. A7416, LLOYD JONES Collection ; Wenlock Limestone, Perlieu Lane, Malverns (figs. 25 and 33 ; fig. 49, Plate 30).

Specimens Examined—A 7416-20, from the type locality ; A6423, A6449-50, A7415, from the Wenlock Limestone of Wenlock Edge ; A5548, from the Wenlock Limestone of Dudley.

Diagnosis—Slender, simple, or dendroid *Tryplasma*, with long rhabdacanthine septa, and numerous thin, concave tabulae extending completely across the lumen.

General Description—The corallum is typically simple and ceratoid, curved at first but erect later, showing repeated cone-in-cone rejuvenescence (fig. 33). Some individuals are dendroid, and increase is peripheral and parricidal. The diameter attained is from 4 to 8 mm, and height as much as 25 mm. The epitheca shows well-marked double-ribs, and fine transverse striation. The acanthine septa are crowded ; there are 21 of each order in a diameter of 4 mm, and 26 in a diameter of 8 mm. The trabeculae of the major septa reach the axis, and those of the minor septa are half, or more than half, this length. The peripheral stereozone is almost as wide as the length of the minor septa. The tabulae are thin, numerous, somewhat irregularly spaced, from 2 to 10, but usually 8 in a space of 2 mm, and concave (fig. 25). They extend completely across the lumen, and rarely show an axial concave notch.

Septal Structure—Each septum consists of a vertical series of rhabdacanths, which are directed from the epitheca up towards the axis at an angle of 45°. Frequently the trabeculae are discontinuous, particularly towards the axis, beginning again on the top of each new tabula. They are always perpendicular to the tabula on which they arise, or through which they pass. The axial ends of the trabeculae of the major septa are not embedded in lamellar sclerenchyme like the obviously rhabdacanthine peripheral ends, but attain only half the diameter of the latter, and do not show rods, so that they appear holacanthine. In the interseptal loculi the lamellar sclerenchyme is laid down parallel with the tabulae, and the stereozone is frequently discontinuous below a tabula.

Variability—The species may be simple or dendroid. The length of the septa is variable ; in those coralla with short septa the tabulae are thicker and further apart, and the portion inside the stereozone is flat, as in *T. loveni*. The number of septa varies with the diameter. The individuals from Wenlock Edge are more slender than those from Malvern, and may be fragments from phaceloid coralla.

Morphology and Histology—The tabulae are of the type supposedly due chiefly to rejuvenescence, as described under *T. loveni*.

Phylogeny—It seems possible that this species was derived from *T. loveni* by a change of shape from trochoid to ceratoid, a lengthening of the septa, an increase in the number of tabulae, and the formation of a compound corallum. It has a

smaller number of septa, in accordance with its smaller diameter, which, in turn, may be correlated with the attainment of a ceratoid corallum. The coralla with the greatest diameter approach closest to *T. loveni* in all other characters also; thus they have shorter septa, and a greater number of the flat tabulae which do not pierce the stereozone and are distantly placed.

Probably the species gave rise to phaceloid forms.

In the Wenlock Limestone there are other simple Tryplasmids which, owing to lack of material, cannot yet be grouped into species, or placed in relation to the phylogenetic series described above.

Tryplasma rugosum (EDWARDS and HAIME)

Eridophyllum (?) *rugosum* EDWARDS and HAIME, 1851, p. 425, Plate x, figs. 4, 4a, 4b.

Tryplasma rugosum (EDWARDS and HAIME); SMITH and LANG, 1927, p. 306, Plate vi, figs. 1-7 (which see).

Specimens Examined—Four corallites from the Lower Wenlock Limestone, Daw End, Walsall; A. J. BUTLER Collection.

General Description (of Daw End specimens) —The corallum is phaceloid, having very slender corallites of average diameter 4 mm (in the holotype from Gotland, the only other specimen known to me, the average diameter is 3 mm), with connecting processes. About 20 major septa are developed, of unequal lengths, and distant from one another. Minor septa (absent in the holotype) were observed in only one corallite, where they were very short. There is a narrow peripheral stereozone. The tabulae are complete, flat, and about 1 mm apart.

Septal Structure (of Daw End specimens) —The septa are rhabdacanthine, the rhabdacanths being directed steeply upwards and towards the axis of the corallum. The "rods" of each rhabdacanth are long, and lie almost parallel to the axis of the rhabdacanth. The stereozone is of lamellar sclerenchyme, continuous with the lamellae of the rhabdacanthine septa.

The absence, or very rare occurrence, of minor septa differentiates this species from all other *Tryplasma*, and its phylogeny is not yet known.

Tryplasma flexuosum (LINNAEUS)

Madrepora flexuosa LINNAEUS, 1758, p. 796.

Tryplasma flexuosum (LINNAEUS); LANG and SMITH, 1927, p. 464, Plate xxxiv, figs. 1a, 1b, 1c; text-figs. 11 and 12 on p. 461 (which see).

Specimens Examined—S.M. A5554, Wenlock Limestone, Dudley; A8168, Wenlock Limestone, Lilleshall Quarry, Wenlock Edge; several from Lower Wenlock Limestone, Daw End, Walsall, A. J. BUTLER Collection.

General Description—The corallum is phaceloid with slender, flexuous corallites, of average diameter 4 mm, and connected by processes. The 20 minor septa are half as long as the major septa. Usually the acanthine septa are very short, the major septa projecting only slightly from a narrow peripheral stereozone. The tabulae are 1 mm apart, flat, unthickened, and sometimes notched at the axis.

Septal Structure—The septa are holacanthine, *i.e.*, the trabeculae are all holacanth, and are set in a continuous lamellar sclerenchyme, which lines the epitheca, thus forming a stereozone. This was the type of septum described by VON KOCH (1882, *b*). The holacanth, is directed inwards, and only slightly upwards.

In S.M. A8168 holacanth, is often developed on the tabulae, so that in some transverse sections the septa appear to extend to the axis of the corallum. The phylogeny of the species is unknown.

Genus *Cystiphyllum* LONSDALE

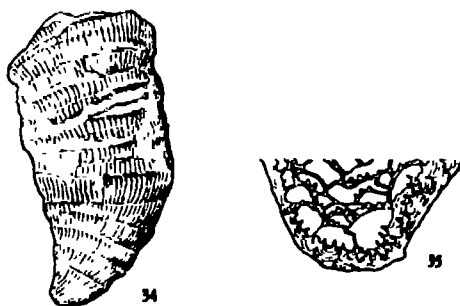
Cystiphyllum LONSDALE, 1839, p. 691.

Cystiphyllum LONSDALE ; LANG and SMITH, 1927, p. 455.

Cystiphyllum densum sp. nov. (Figs. 34–35 ; figs. 50, 51, Plate 30.)

Holotype—S.M. A6455, from the Wenlock Shale of the Malvern Tunnel tip-heap, Colwall Station.

Specimens Examined—Topotypes, S.M. A7377–81, 6853 ; 7376, from the Wenlock Shale of Titford Shaft, 400 yards down, South Staffordshire Coalfield. A7382–5, from the Wenlock of BARBER'S Quarry, Falfield, near Tortworth, Gloucester.



FIGS. 34–35—*Cystiphyllum*

FIG. 34—Turbinately cylindrical corallum of *C. densum*, showing rejuvenescence, natural size.

FIG. 35—Section showing domed plates of horizontal tissue, bearing holacanth, and consisting of lamellar sclerenchyme.

Diagnosis—Short, curved, trochoid *Cystiphyllum*, with much lamellar sclerenchyme proximally.

General Description—The corallum is trochoid and gently curved at first, becoming a cylinder with an average diameter of 20 mm, and a height of 30 mm (fig. 34). Cone-in-cone rejuvenescence is common. The epitheca shows fine longitudinal

and transverse striation. Specimens whose epitheca has been destroyed show vertical series of minute holes, from which the septal spines have been weathered away. The septa are very numerous and long, 6 in the space of 3 mm. Each septum is represented by a vertical series of short, slender spines, based on the epitheca and horizontal tissue, and directed upwards and towards the axis, rarely penetrating the succeeding horizontal skeletal elements. Major septa cannot be distinguished from minor septa in transverse section. The horizontal skeletal elements are copiously developed, but cannot well be differentiated into dissepiments and tabulae. The peripheral plates are domed, varying in size, and steeply inclined towards the axis, each extending over several interseptal loculi. The axial plates are incomplete and domed, and are less steeply inclined, and rather larger, than the peripheral plates. Lamellar sclerenchyme is always deposited in the apex, and sporadically in the upper parts of the corallum.

Septal Structure—The septa are holacanthine, each consisting of one vertical series of holacanthi. They are discontinuous, for a new set of holacanthi arises on each successive horizontal skeletal element. The holacanthi always lie in the vertical plane of the septum, and perpendicular to the curve of the horizontal tissue (fig. 35). Those of one vertical series are about 0.4 mm, apart, but those of neighbouring septa are 0.5 mm, apart. Some thin sections indicate that the holacanthi are formed of fibres directed radially and upwards from the axis. The horizontal skeletal elements consist of more or less thick deposits of lamellar sclerenchyme, as in *Tryplasma primum*.

Variability—The coralla vary considerably in the length of the cylindrical portion. The size and relations of the horizontal skeletal elements vary within wide limits, and much or little lamellar sclerenchyme may be present.

Morphology and Histology—In this species the horizontal tissue consists entirely of domed plates, each formed, like the tabulae in *Tryplasma primum*, of a more or less thick deposit of lamellar sclerenchyme. The first lamella of each plate is laid down after a sudden uplift of part of the ectoderm of the base of the polyp; auxiliary lamellae are then deposited, and are usually continuous in neighbouring plates. The sudden uplift affects an almost circular area of the base of the polyp, and the central part of this rises furthest, the ectoderm of the edges remaining attached. Typically a new plate has its maximum curvature above the point of junction of the plates on which it is based. This is doubtless governed by tension in the base of the polyp. The holacanthi are well developed only where there is a considerable thickness of lamellar sclerenchyme. They must have arisen in permanent, shallow, pointed invaginations, arranged in radial series over the base of the polyp.

Phylogeny—The lamellar sclerenchyme and the holacanthine septa suggest relationship with the *Acanthocyclus-Tryplasma* group. But the copious development of the domed plates, of horizontal skeletal tissue (which may be present even in the apex), and the entire absence of rhabdacanthi are against any very close relationship, although the speed of evolution may have suppressed the links. The Cystiphyllids already known from the Upper Llandovery, the Woolhope Limestone,

the Wenlock Shale, the Wenlock Limestone, and the Lower Ludlow Shale probably form a cognate group, but their ancestors and descendants are as yet unknown. In connexion with *Cystiphyllum* it may be mentioned that Dr. STANLEY SMITH has shown me a series of slides showing that the Devonian *Cystiphyllum vesiculosum* was derived from a form with well-developed lamellar septa, pinnately fibrous in transverse section. A quick distinction between Silurian and Devonian Cystiphyllids may be made by observing that the dilating sclerenchyme of the former is dominantly lamellar, while that of the latter is dominantly fibrous, the fibres having their long axes at right angles to the surface of a dissepiment or tabula.

Cystiphyllum siluriense LONSDALE

Cystiphyllum siluriense LONSDALE, 1839, p. 691, and Plate xvi *bis*, figs. 1, 1a, non fig. 2. Wenlock Limestone; Wenlock and Dudley.

Cystiphyllum siluriense LONSDALE; LANG and SMITH, 1927, p. 455, 476.

Cystiphyllum cylindricum LONSDALE. (Figs. 52 and 53, Plate 30.)

Cystiphyllum cylindricum LONSDALE, 1839, p. 691 and Plate xvi *bis*, figs. 3, 3a-b; Wenlock Limestone; Benthall Edge.

Cystiphyllum cylindricum LONSDALE; LANG and SMITH, 1927, pp. 455, 477, Plate xxxvi.

I have been unable to discover that these two species are distinguished by any constant difference in internal structure. They are therefore described together. *C. siluriense* is typically a large turbinate form, while *C. cylindricum* is small and cylindrical. They may show cone-in-cone rejuvenescence, root-processes, or foot-like outgrowths from the basal curved part (fig. 53, Plate 30). In their internal structure they differ from the above-described Wenlock Shale form, from which they have probably descended, only in that lamellar sclerenchyme is not developed at the apex.

Genus *Cantrillia* SMITH

Cantrillia SMITH, 1930, p. 298.

Genotype (by designation): *Cantrillia prisca* SMITH, 1930, p. 298.

Cantrillia prisca SMITH

Cantrillia prisca SMITH, 1930, p. 298, Plate xxvi, figs. 9-19, and Text-fig. 2; Upper Llandovery, Purple Shales; Hughley, Shropshire.

Specimens Examined—S.M. A7421-3, from the type locality; A7424-8, from the Upper Llandovery of the Malvern tunnel tip-heap at Colwall Station.

General Description—The corallum is small, simple, and curved-ceratoid, with an average diameter of 4 mm, and an average length of 15 mm. There are about 60

septa in two orders, each septum being represented by a vertical series of trabeculae set in a wide zone of sclerenchyme, which lines the epitheca. A single infold of the lining tissue is characteristic. The tabulae are thick, horizontal, and distant.

Septal Structure—The trabeculae are usually holacanth, but the specimens from Colwall show rhabdacanth. The sclerenchyme lining is lamellar, bearing the same relation to the trabeculae as in *Tryplasma primum*.

Discussion—The development of the trabeculae is variable, and the in-folding may be prominent or indistinct. The fold must be the result of a persistent tuck in the base of the polyp. The species is earlier than the above-described members of the *Acanthocyclus* group, to which the trabeculae and the lamellar sclerenchyme suggest relationship. The fold in the lining differentiates it from all other forms.

CONCLUSIONS

From the foregoing descriptions certain conclusions may be drawn.

Systematics—Acanthine septa with large spiniform trabeculae of nearly equal size are found in a number of British Silurian corals. These corals belong to the genera *Palaeocyclus*, *Acanthocyclus*, *Tryplasma*, *Cystiphyllum*, and *Cantrillia*, and probably form a cognate group here called the Acanthocyclusidae. It is possible to recognize the genera and species of this group by their septal structure.

Variability—Most of the variations observed in this group are stages in the trends of development (discussed below) by which it was evolving. But one or two are not concerned in the immediate evolution of the group. Such are the cross-bar nature of some of the spines of *P. porpita*, and the occurrence of two series of septal spines in some septa of *A. porpitoidea*. These are expected to have significance in the discussion of other types of septal structure.

Morphology and Histology—It can be safely assumed that the skeleton of a Rugose coral was secreted by its polyp in the same way as that of a Recent coral. That is, it was laid down as an exo-skeleton of calcareous fibres by the ectoderm of the base of the polyp, vertical skeletal elements being formed in radial invaginations, and horizontal skeletal elements being secreted by the unfolded ectoderm between the invaginations (figs. 1–7).

In this group the only vertical skeletal elements formed are the *septa*. As shown by PRATZ (1882, p. 88) and OGILVIE (1897, p. 124), the septa consist of trabeculae. There are three types of trabeculae in this group (figs. 8–10).

(1) A *monacanth* consists of curved fibres directed radially upwards and outwards from the axis. It has an average diameter of 0.5 mm.

(2) A *rhabdacanth* consists of straight "rods" directed radially upwards from its axis. It also has a diameter of 0.5 mm. Each "rod" probably consists of straight fibres directed radially upwards from its axis, and has a diameter of 0.05 mm. Rhabdacanthi are always bound together by lamellar sclerenchyme.

(3) A *holacanth* probably consists of straight fibres directed radially upwards from its axis. Its diameter is about 2 mm, and it is usually surrounded by lamellar sclerenchyme.

Four types of acanthine septa are found in this group (figs. 14–17) :—

(1) A *monacanthine* septum is formed of contiguous monacanths ; ex. *Palaeocyclus porpita*.

(2) A *rhabdacanthine* septum is formed of contiguous rhabdacanths bound into a plate by lamellar sclerenchyme. Neighbouring rhabdacanthine septa are bound together by the lamellar sclerenchyme ; ex. *Acanthocyclus porpitoides*.

(3) A *dimorphacanthine* septum is formed of a vertical series of rhabdacanths and holacanths separately embedded in lamellar sclerenchyme, which also binds together neighbouring septa ; ex. *Tryplasma primum*.

(4) A *holacanthine* septum is formed by a vertical series of holacanths, each separately embedded in lamellar sclerenchyme ; ex. *Tryplasma flexuosum*. Holacanthine septa may be discontinuous, the holacanths being deposited anew on successive horizontal skeletal elements ; ex. *Cystiphyllum siluriense*.

The general character of the septal invagination in the base of the polyp is the same throughout the group. There are pointed invaginations about 5 mm apart along its summit. Deposition of each trabecula is begun by the ectoderm at the apex of a pointed invagination, and continued by the lateral ectoderm (figs. 11–13). The different types of trabeculae are caused by changes in the secretory functions of the lateral ectoderm. To form a monacanth all the lateral cells (or parts of the syncytium) add fibres without noticeable arrangement in growth-lamellae. To form a rhabdacanth some of the lateral cells (or parts of the syncytium) remain as subsidiary invaginations, each of which forms a rod, while the intervening cells lay down fibres arranged in obvious growth-lamellae. To form a holacanth, all the lateral cells (or parts of the syncytium) lay down fibres arranged in growth-lamellae. That an invagination for a monacanth may change to that for a rhabdacanth is indicated by the occasional suggestion of rods in a monacanth ; that the invagination for a holacanth can easily replace the invagination for a rhabdacanth is proved by the occurrence of both types in the one radial invagination.

Horizontal tissue (figs. 18–25) is developed in all members of this group, except *Palaeocyclus porpita*. It is always of lamellar sclerenchyme, i.e., it is obviously divided into growth-lamellae. Each growth-lamella probably consists of fibres with their long axes at right angles to its surfaces. Three arrangements of the lamellae are known in the group. (a) Lamellae are superposed directly on the top of one another throughout the corallum. In the thicker axial deposits of conical coralla, cross-bedding of the lamellae occurs. Ex. *Acanthocyclus*. (b) Spaces occur between the lamellae in the axial zone, causing the lamellae to be grouped into flat or sagging tabulae ; ex. *Cantrillia* and *Tryplasma*. (c) Domed spaces occur between the lamellae throughout the corallum, causing the lamellae to be grouped into domed horizontal skeletal elements ; ex. *Cystiphyllum*. The formation of the spaces is

thought to be due to movements of the base of the polyp to relieve pressure in it or upon it. The chief source of this pressure is the difference in vertical growth between the vertical and horizontal skeletal elements. In some cases rejuvenescence coincides with, and may cause, tabula-formation.

Phylogeny—The lineage *Acanthocyclus porpitoides*—*A. fletcheri*—*A. transiens*—*Tryplasma primum*—*T. loveni*—*T. malvernense* is well substantiated, each species being connected with the next by transients. The changes involved were (a) a change in shape from discoid through patellate, turbinate, and trochoid, to ceratoid (figs. 27–33); (b) an evolution in the horizontal tissue (figs. 19–25); more lamellar sclerenchyme is deposited; it becomes cross-bedded axially, and axial spaces arise, giving primitive tabulae which become regularized; (c) The rhabdacanthine septa degenerate to dimorphacanthine septa, but revert in *T. loveni* and *T. malvernense*, which also may become dendroid. The stratigraphical evidence, which is, however, imperfect, supports this hypothesis of descent. The first four species are from the Wenlock Shale, and the last two from the Wenlock Limestone. Parts of this evolution were noted by LINDSTRÖM (1882, p. 66) in specimens from Gotland.

These trends of development are orthogenetic. For in the Lower Ludlow there also occurs a variable group, which is more patellate than *A. transiens*, and varies in internal structure from an advanced *A. fletcheri* stage to an early *T. primum* stage. In the Wenlock shale the erect forms of *A. fletcheri* may give rise to turbinate, Tryplasmoid coralla, without passing phylogenetically through an *A. transiens* stage. Thus *Tryplasma* has been derived more than once from *Acanthocyclus*; and the species *T. primum*, the Tryplasmoid individuals arising direct from *A. fletcheri*, and the Ludlow *A. aff. transiens*, illustrate very well the concept of genomorphs (SMITH and LANG, 1930, p. 179).

Recapitulation in this group is very quickly concluded. Thus, while *Tryplasma primum* still has most of its apex filled with lamellar sclerenchyme, showing its origin from *Acanthocyclus transiens*, its descendant *T. loveni* has no more lamellar sclerenchyme developed at its apex than on its sides.

A possible ancestor to *A. porpitoides* is *Palaeocyclus porpita*, from the Upper Llandovery of the Welsh Borderland, the (?) Woolhope of Pembrokeshire, and the Lower Visby marls of the Gotland succession. A change from the flat disc of *P. porpita* to the more patellate disc of *A. porpitoides* would be the first step in the shape-change. Nothing corresponding to horizontal tissue is present in *P. porpita*, but the appearance of lamellar sclerenchyme lining the epitheca in the interseptal loculi of *A. porpitoides* may be regarded as the first step in the evolution of horizontal tissue. Thirdly, it is not impossible to derive the rhabdacanthine septa of *A. porpitoides* from the monacanthine septa of *P. porpita*, this being the first and greatest step in the degeneration of the septa.

The phylogeny of other simple and compound Tryplasmids from the Wenlock Limestone is as yet untraced.

Cantrillia must be considered a member of the Acanthocyclidae because of its dimorphacanthine septa, lamellar sclerenchyme, and primitive tabulae.

Cystiphyllum is probably closely related to the *Acanthocyclus-Tryplasma* group. This is suggested by the holacanthine septa and lamellar sclerenchyme. But horizontal tissue is copiously developed as domed plates, and a small trochoid *Cystiphyllum* is already present in the Upper Llandovery Purple Shales of Shropshire.

ACKNOWLEDGMENTS

In writing this paper I have received a great deal of help from Dr. W. D. LANG, to whom I am also deeply indebted for his constant inspiration and encouragement. Dr. D. E. INNES was kind enough to send me his sections and notes on the septal structure of this and other groups. Dr. STANLEY SMITH, Dr. H. DIGHTON THOMAS, and Mr. A. G. BRIGHTON most helpfully criticized the manuscript; and I have had much inspiration from discussion with Miss F. E. S. CALDWELL and Mr. A. J. BUTLER. To Miss G. L. ELLES I am particularly indebted for advice and encouragement at all times. Some of the drawings were done by Miss E. TALBOT, and Miss E. RIPPER gave me help with others. I wish to thank Dr. W. D. LANG and Dr. H. D. THOMAS, Mr. A. G. BRIGHTON, Professor PRIVETEAU, and Professor GERMAIN, for facilities at the British Museum (Natural History), Sedgwick Museum, Cambridge, École des Mines, Paris, and Musée d'Histoire Naturelle, Paris. The work was done during my tenure of the Old Students' Research Fellowship of Newnham College, Cambridge.

SUMMARY

Research into the minute septal structure of the Silurian Rugose Coral genera *Palaeocyclus*, *Acanthocyclus*, *Tryplasma*, *Cystiphyllum*, and *Cantrillia*—genera in which the septa are essentially acanthine—shows that the ultimate septal structure is "fibrous", and that the crystalline fibres are either aggregated into spines, or form layers of sclerenchyme. The genera just named probably form a cognate group. In *Palaeocyclus* the septa are continuous plates, formed of contiguous spines, but in *Acanthocyclus* and *Tryplasma* the spines are more or less obviously discrete. The minute septal structure points to an evolution from *Palaeocyclus*, through various species of *Acanthocyclus* to *Tryplasma*, and the passage from *Acanthocyclus* to *Tryplasma* has occurred along more than one lineage, indicating orthogenetic trends. In *Palaeocyclus* the spine (trabecula) is a simple bundle of crystalline fibres, and is here termed a *monacanth*. In *Acanthocyclus* each spine is formed of an aggregate of rod-like bodies, each of these being formed of a bundle of crystalline fibres. The ends of the rods often project from the surface of the spine, giving it a prickly appearance. Such spines are here called *rhabdacanth*s. Again, in some species of *Acanthocyclus* and in *Tryplasma* certain of the spines are *rhabdacanth*s, and others are about half the diameter of the *rhabdacanth*s and show no sign of "rods". They occur exclusively in *Cystiphyllum*. Such spines are here called *holacanth*s. It is suggested how each of these types of minute septal structure can have been related to the secretory surface of the soft parts.

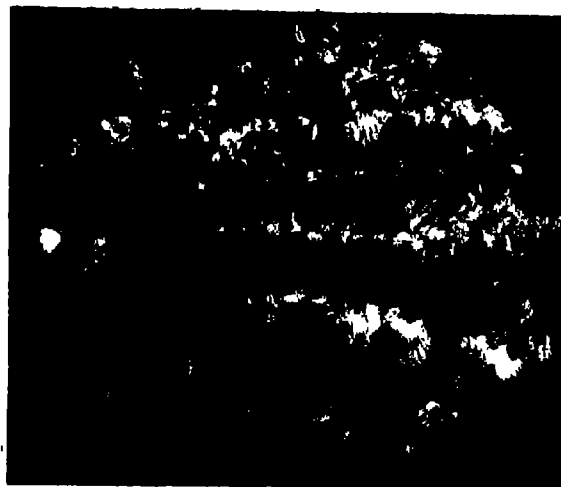
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EXPLANATION OF PLATES

PLATE 29

- FIG. 36—*Palaeocyclus porpita* (LINNÆUS). B.M. R25556, specimen 2, from the Lower Visby Marls, Gotland. Part of transverse section in lower part of corallum at the axial ends of the minor septa. The septa (*s.*) are separated by original sutures (*su.*) and are seen to consist of fibrous monacanth, whose axes of calcification are seen at *ax.* No horizontal tissue is developed. $\times 40$ diameters.
- FIG. 37—*Acanthocyclus porpitoides* (LANG and SMITH). S.M. A6286, Wenlock Shale, Dudley. Part of transverse section in lower part of corallum, at the axial ends of the minor septa. The septa (*s.*) are separated by horizontal tissue in form of lamellar sclerenchyme (*lam.*). The rhabdacanth (*rh.*) of the septa are seen, each consisting of "rods" in the lamellar sclerenchyme. $\times 40$ diameters.
- FIG. 38—*Acanthocyclus* aff. *transiens* sp. nov. S.M. A6439, Lower Ludlow Shales, Ledbury Quarry. Rhabdacanth (*rh.*) are seen in vertical section, which is median at the top, and becomes tangential lower, so that the "rods" (*r.*) are in vertical section at the top of the figure and in transverse section below. *lam.* lamellar sclerenchyme. Cf. fig. 15C. $\times 40$ diameters.
- FIG. 39—*Tryplasma primum* sp. nov. An enlargement ($\times 40$ diameters) of a tabula, in which the lamellae of the sclerenchyme are arranged *en echelon*.
- FIG. 40—*Acanthocyclus transiens* sp. nov. S.M. A6437c, Wenlock Shale, Malvern tunnel tip-heap, showing cross-bedding of the lamellar sclerenchyme near the apex (cf. fig. 22). Holacanth (*h.*) are seen; *a. b.*, air bubble in section. $\times 15$ diameters.



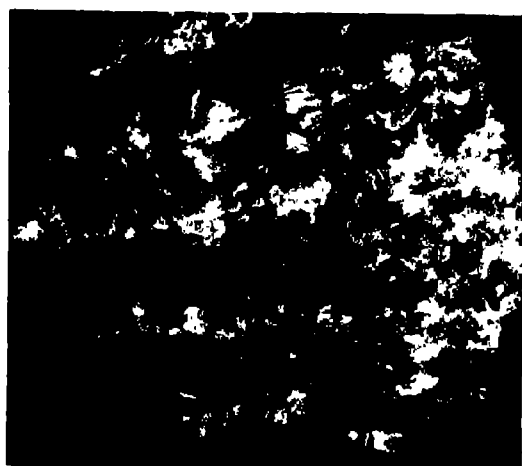
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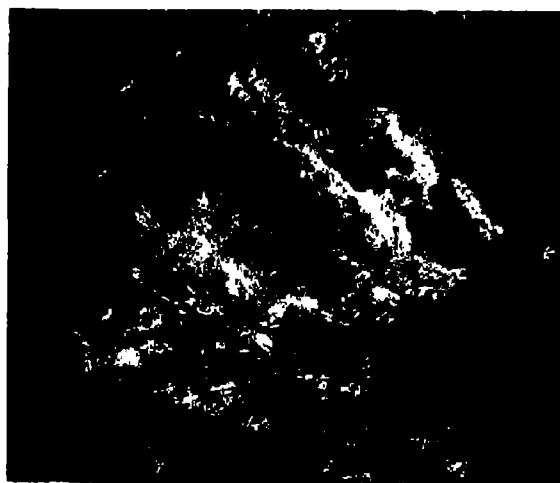
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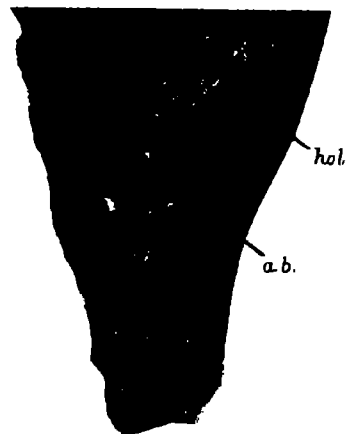
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38

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40

PLATE 30

(All figures $\times 2$ diameters.)

- FIG. 41—*Acanthocyclus transiens* sp. nov. S.M. A6851c, Wenlock Shale, Malvern tunnel tip-heap, Colwall Station. Median vertical section. Shows lamellar sclerenchyme and holacanthus (*hol.*).
- FIG. 42—*Acanthocyclus transiens* sp. nov. S.M. A5936C, Wenlock Shale, Dudley. Transverse section through bottom of calice. Shows rhabdacanthus cut obliquely (*cf.* fig. 15B).
- FIG. 43—*Tryplasma primum* sp. nov. S.M. A6445C, Wenlock Shale, Malvern Tunnel tip-heap, Colwall Station. Median vertical section. The tabulae are of thick lamellar sclerenchyme, and the septa are holacanthine.
- FIG. 44—*Tryplasma primum* sp. nov. Paratype. S.M. A6442c. Specimen transitional from *A. transiens*. Median vertical section.
- FIG. 45—*Tryplasma primum* sp. nov. Paratype. S.M. A5707b. Transverse section. The axis is occupied by a tabula.
- FIGS. 46-47—*Tryplasma loveni* (EDWARDS and HAMR). S.M. A6448d and e, Wenlock Limestone, Knowle Quarry, Prethope, Wenlock Edge. The septa are rhabdacanthine, and the tabulae are notched. 46, transverse section; 47, median vertical section.
- FIGS. 48-49—*Tryplasma malvernense* sp. nov. Wenlock Limestone, Perlieu Lane, Malvern. 48, S.M. A7420c, transverse section; 49, S.M. A7416b, median vertical section. Holotype, *see* figs. 25 and 33A.
- FIGS. 50-51—*Cystiphyllum densum* sp. nov. 50, Holotype, S.M. A6455c, in median vertical section. Wenlock Shale, Malvern Tunnel tip-heap, Colwall Station. 51, S.M. A7376c, Wenlock Shale, Titford Shaft, South Staffordshire Coalfield, in transverse section.
- FIGS. 52-53—*Cystiphyllum cylindricum* LONSDALE. S.M. A6453d, e, Wenlock Limestone, Knowle Quarry, Prethope, Wenlock Edge. 52, transverse section; 53, median vertical section.



hol.

41



45



50



42



46



51



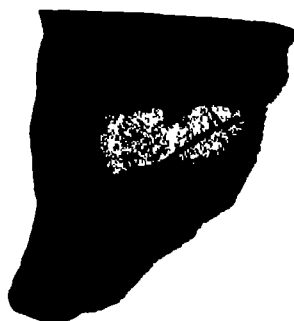
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53

VII—On the Factors which Influence the External Form of Fossil Plants; with Descriptions of the Foliage of Some Species of the Palaeozoic Equisetalean Genus *Annularia* Sternberg

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(Communicated by A. C. SEWARD, For. Sec. R.S.—Received January 14—Read May 21, 1936)

[PLATES 31 AND 32]

I—ON THE FACTORS WHICH INFLUENCE THE EXTERNAL FORM OF FOSSIL PLANTS

Most writers of general treatises on Palaeobotany give an introductory chapter on fossilization, and this as a rule includes an account of the various kinds of fossil plants and the circumstances under which plants may become incorporated in sediments. The differences between the external form of the fossil and the original plant fragment are not often mentioned in relation to the factors which operate in the production of a fossil. It is often implied that fossil plants of the "incrustation" or "impression" type are produced in much the same way as herbarium specimens. That such a crude analogy does not satisfactorily explain the forms found among fossil plants of this type is obvious to those who have applied transfer methods to the examination of such fossils. In the writer's opinion, the reason why so little precision has been given to descriptions of fossil-plant forms is that insufficient attention has been paid to the properties of sediments and the role played by the matrix. While many writers recognize that the differences in shape between a fossil and the original plant are due to the compressibility of the plant tissues the writer is not aware of any reference in the literature to the importance of the compressibility of the matrix in determining the form of a fossil embedded in it. It will be shown that the alteration in shape of a plant in undergoing fossilization in many cases may be best described as the result first of the collapse of the plant tissues and second of a more or less uniform vertical strain of the fossil and the surrounding matrix together.

The relative compressibility of the sedimentary matrix and the plant substance in a mass such as coal has been realized as an important factor by KENDAL (1918, p. 463), who has used it to explain the form of shale or sandstone "partings" in coal-seams. As the result of his study of these structures he was led to assume "that in the consolidation of the coal-bearing strata coal itself—in the change from the condition of peat to the hard mineral—undergoes a very much greater reduction in volume than does a sand bed or well compacted mud bed". He gives good reasons

for believing that in the transformation from peat to coal there may be a compression to one-twentieth the original thickness (KENDAL, 1923, p. 65).

In the discussion of KENDAL's theories it has been suggested by one geologist (*see under* KENDAL, 1918, p. 476) that water-logged peat could not possibly be compressed to such an extent for water is practically incompressible. The incompressibility of water has, however, no bearing on the question, for the mud or sand matrix is porous and the water is probably free to rise up between the particles. The layers of sediment overlying a coal seam may be most aptly compared with a porous piston in which the pores are sufficiently large for the water to escape from the compression chamber but not sufficiently large to allow the particles of sand or mud to escape.

KENDAL (1923, p. 63) points out that freshly deposited mud may contain 90% water and when reduced to the condition of shale may still contain 20% interspace. Thus, according to him, a mud may be reduced at least to one-third its original thickness. On the other hand, a bed of sand deposited in water may suffer scarcely any loss in bulk once it has passed the quicksand stage. Obviously mixtures of sand and mud will have contractions which range between these limits, and muds with a high percentage of organic content will exhibit even greater contractions.

KENDAL (1923, p. 65) showed that owing to the differential compressibility of plant substance and matrix the shape of a mass of sediment enclosed in a coal seam may undergo notable changes in form.

It will be shown that similar principles may be applied to the explanation of the form of fossil plants which may often be regarded as small coal seams enclosed in a sandy or shaly matrix.

Fossil plants may be classified as follows :—

1 *Petrifactions*—(Plantes minéralisées, ZEILLER 1900, p. 9.) The external form, the internal cellular structure, and sometimes the carbonaceous substance of the original plant, is preserved, *e.g.*, coal-balls, silicified plants.

2 *Incrustations* in a restricted sense ("Moulages," ZEILLER, 1900, p. 11). The external form of the plant is preserved in the form of a cast. The internal structure is not preserved and usually the carbonaceous substance is completely replaced by inorganic matter, *e.g.*, casts of plants in tuffs (ZEILLER, 1900, pp. 10, 11).

3 *Compressions*—External form of the plant modified by the vertical pressure of the sediment with which it is surrounded. Vertical dimensions of the plant fragment reduced, but the horizontal dimensions usually unchanged. The substance of the plant is usually preserved in the form of coaly matter which sometimes retains some of the original cutinized membranes and a certain amount of the original structure.

4 *Impressions*—The form impressed by the fossil plants of the incrustation and compression type on the matrix are usually termed impressions.

5 *Compactions*—Masses of plant fragments without intervening matrix such as are found in peat. They are compressed by vertical pressure and *deformed by the pressure of one fragment against another*. *E.g.*, Peat, lignite, and coal in seams. A large proportion of the solid substance of the plant is preserved in the form of carbonaceous

residues and may retain some of the original membranes and a certain amount of structure.

This discussion deals with the compression-type of fossil plant. Most fossil plants are in the form of thin sheets of carbonaceous matter which when viewed normally to the bedding plane bear a very close resemblance to the original leaves, stems, roots, and fructifications, but which are, as regards relief, profoundly modified. This type of fossil is usually found in sandstones or shales which have been formed by the consolidation of sediment under its own weight in water. Fossil plants of this type, for which I suggest the name *compressions*, have in the past been named plant-impressions, Pflanzenabdrücken, or impressions végétales. Several palaeobotanists have pointed out the misleading nature of these terms, and Dr. H. HAMSHAW THOMAS has suggested the use of the description mummified plants. While mummified plants is perhaps the best expression so far proposed, it does not suggest the considerable distortion in form which is characteristic of this type of fossil. In a mummy the alteration is generally in the nature of a uniform shrinkage due to the drying up of the soft tissues.

A Theory to Account for the Form of Fossil Plants of the Compression-type

It may be supposed that in the formation of fossil plants of the *compression-type* the plant fragments are embedded in silt or sand under the surface of the water. It is obvious that a plant fragment for dynamic reasons will come to rest in a position in which its centre of gravity is lowest and its shortest axis vertical. The most extended surface will be extended in the bedding plane.

The plant fragments are then covered and surrounded by further deposit of mud. The rate at which this deposit occurs will undoubtedly influence the form of the fossil. If the rate of covering is slow, oxidation and decay processes may remove a large part of the less resistant tissues and little but the cuticle may remain. If sedimentation is rapid, the loss of organic substance is checked, and if the mud contains fine particles such as clay the plant fragments may be very effectively sealed up and their organic substance retained within the epidermal cuticle.

Before considering the further changes that may take place in the form of the plant fragments, it is necessary to consider the changes that take place in the mud. As KENDAL has shown, freshly deposited mud may contain as much as 90% water and may be reduced by partial elimination of its water to one-third its original thickness. This contraction of the mud may be produced by drying, but under the conditions postulated here in a mass of mud of indefinite horizontal extent the water is removed by displacement upwards. If we consider an average particle of solid material in the mud and the forces acting on it we see that the sum of the horizontal components of the forces acting on it due to the water and the surrounding particles is zero. If there is a clear water space below it will sink owing to its own weight in water and the pressure due to particles weighing on it above. Its resultant movement will be a vertical downward movement and water is displaced upwards.

If we now consider the mud deposit as a whole we may regard the solid part of the mud as a sponge. The mud is deposited in a basin formed by an estuary or lake the sides of which support the mass, and any lateral thrust forces are taken up and counterbalanced by the backward pressure of the sides of the basin. Under these conditions it is clear that a spherical mass of sediment will, as it is buried deeper and deeper, assume the shape of a spheroid and, moreover, the major axis of the spheroid will be horizontal and equal to the diameter of the original sphere (fig. 1) as there can be no lateral displacements.

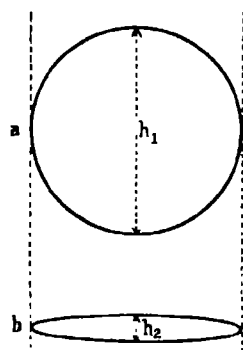


FIG. 1—Diagram to illustrate the change in shape of a spherical portion of a large deposit of mud in an estuarine or lacustrine basin. The change in shape is due to the weight of the spongy mass of solid particles and to the upward displacement of water. The sphere becomes spheroidal in shape and its major diameter is horizontal and is equal to the original diameter of the sphere.

It is probable that a mud may be compressed ultimately to considerably less than one-third of its original thickness. If the mud consists largely of vegetable particles the first water to be displaced upwards will be the water held in the interspaces between the particles, then as the pressure increases one would expect that the water held by the colloidal particles would tend to be forced out and finally there would be a loss of bulk from the vegetable carbohydrates as the vegetable material approached coal in character and contained a greater proportion of hydrocarbons.

If a relatively large organic fragment such as a leaf is embedded in mud its shape is altered as the mud becomes converted in course of time into shale. From a study of fossil plants in shale it is obvious that the dimensions of the plant fragments normal to the bedding plane are considerably reduced. There is usually no evidence of alteration of dimensions in directions parallel to the bedding plane. This is what one would expect if the plant fragment possessed the same spongy properties as the mud in which it was embedded. As a matter of fact, a leaf may contain as much as 90% of water, and even a relatively solid plant tissue such as wood is exceedingly porous and, when rotten, spongy in character. We might therefore be led to expect that a spherical fragment of plant tissue embedded in mud would become spheroidal just as the spherical element of mud.

Taking as an example of a woody structure a cylinder of wood we may consider the change in shape that will occur when it is embedded with its long axis horizontal in a compressible matrix. If the compressibility of the wood is the same as that of the matrix, the cylinder will finally have an elliptical section when cut transversely to its axis (fig. 1). The major axis of the ellipse will be equal to the original diameter of the cylinder. The writer has measured a number of lignitized *Gleichenia* rachises from the Cretaceous rocks of Greenland which are elliptical in cross-section. The rachises varied from 3 mm to 15 mm in breadth but the ratio of breadth to thickness in all of them lay between the limits 1:0.14 and 1:0.2. The rachises were

extracted from a mass of fine vegetable debris which would represent a highly compressible matrix.

As another illustration we may take a hollow cylinder of fairly compressible tissue embedded in a less compressible matrix which also fills the hollow of the cylinder. The result which is suggested in fig. 2, *b* is not an unknown type of fossil. The hollow stems of *Calamites*, particularly those with a considerable development of secondary wood, are found in this form (e.g., *Calamites approximatus* BGT. SEWARD, 1898, p. 370, fig. 100). The pith cast covered with a layer of carbonaceous material stands up in relief while at the sides is a border of carbonaceous material representing the wood and other tissues which did not underlie or overlie the pith cavity.

If, on the other hand, we consider the fossilization of a leaf which contains perhaps more than 80% of water we find that another factor comes into play. The examples so far considered have similar upper and lower surfaces when they have acquired their final form. The fossil is convex on both sides and both sides have suffered the same degree of alteration. With a leaf, however, the result is different, the contours of the upper surface are usually more changed than those of the under surface. The soft tissues of the leaf would collapse under the weight of the overlying sediment more readily than the surrounding matrix, and as a result the upper surface of the leaf would sink as the water in the leaf was displaced upwards. Under these circumstances the surface of the matrix underneath the leaf is subjected at first to very little pressure and its form does not alter to any considerable degree during this part of the process. In other words, the upper surface of the leaf subsides as the water is pressed out of it and the substance of the leaf forms a layer over the surface of the matrix which formed a cast of the lower surface of the leaf. (Fig. 3, A and B.) After this stage is reached the compressibility of the concentrated material of the leaf will approximate to that of the matrix and the whole leaf-matrix system may have a uniform compressibility. Subsequent compression due to added deposition of sediment may further change the shape of the leaf by the production of a uniform vertical deformation in the leaf and the surrounding matrix.

The process of fossilization of a leaf from the point of view of its external form may therefore take place in two stages; in the first the leaf collapses on to the matrix surface below it by loss of water, in the second it undergoes with the matrix a practically uniform vertical deformation. The shape of the resulting fossil is principally based on the form of the lower surface of the original plant fragment. Thus the leaf represented in vertical section in the sediment in fig. 3 A₁ first collapses

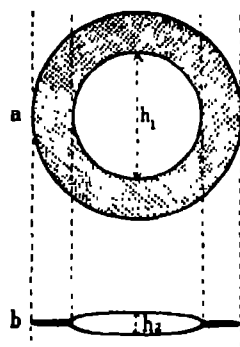


FIG. 2—Diagram to illustrate the approximate result of compression in a sediment of a hollow cylinder or sphere of highly compressible material filled with the sediment. The sediment has undergone a compression to about one sixth its original depth. The core of sediment would only be approximately elliptical in section as the less resistant material round it might allow of some lateral bulging.

to form what is seen in A_3 and then all the vertical dimensions undergo a reduction to about one-quarter, a figure representing the compressibility of the matrix.

It is evident that under the postulated conditions a lateral expansion of the fragment is impossible when the matrix is uniform round about it. The tendency for the fragment to bulge out at the sides under the pressure of the overlying matrix will be prevented by the resistance of the matrix at the sides. The pressure at the sides will always be greater than that on top of the leaf owing to the greater depth at the sides.

The compressibility of a sediment will depend on the size and shape of its particles and the amount of water and compressible organic particles contained in it. A sandy matrix will be relatively incompressible, but a mud or silt containing much water and a high organic content will be very compressible. Corresponding to



FIG. 3.—Diagrams representing the compression in a sediment of a leaf with a concavo-convex lamina. In A_1 the leaf is buried with the convex surface up and in B_1 with the concave surface up. A_1 and B_1 represent the effect of the collapse of the soft tissues as the water in them is displaced upwards and A_3 and B_3 the final form of the fossil after the vertical compression of the sediment is complete. A compression border (c) is formed in A_3 .

these differences in the nature of the matrix we usually find a relatively high degree of relief in fossils preserved in sandy rocks or in rocks with a low percentage of organic or humic material, while in fine muds with a large amount of organic matter the greater compressibility usually results in the fossils having a low degree of relief. This is illustrated in fig. 3, A and B, where it is obvious that the greater the compressibility of matrix the smaller h_3 will be and the flatter the resulting fossil.

The following examples illustrate peculiarities exhibited by some fossil plants of the compression type which may be most readily explained by supposing that fossilization has taken place in the manner suggested by the above theory.

1 *The Shape of Leaf Margins*—In compressions of leaves belonging to the form-genera *Alethopteris*, *Neuropteris*, and *Pecopteris* the lamina of a leaflet was, we must suppose, often convex or concave in shape and there is frequently a narrow, flat border round the margin in the fossils. This border is of the same carbonaceous substance as the rest of the lamina and is surrounded by the cuticle. It is set at an

angle to the curved part of the lamina and may be termed a *compression border*. It will be seen by reference to fig. 3 (A and B) that it is most likely to form if the original leaflet was orientated in the bedding plane with its convex surface uppermost. This border would probably not be formed if the leaflet was situated with the convex surface underneath. If a hand specimen of rock has a leaf on it showing this border it should be possible to determine the upper and lower side of the specimen in relation to the bed of rock from which it had been extracted. If such a fossil were found in position in the rock stratum it would be possible to determine whether the stratum was normally orientated or whether as a result of geological disturbances had been inverted. Moreover, the width of the border on a fossil leaf is a measure of the thickness of the original leaf-lamina.

Another example of a compression border is found in inrolled leaves. The writer

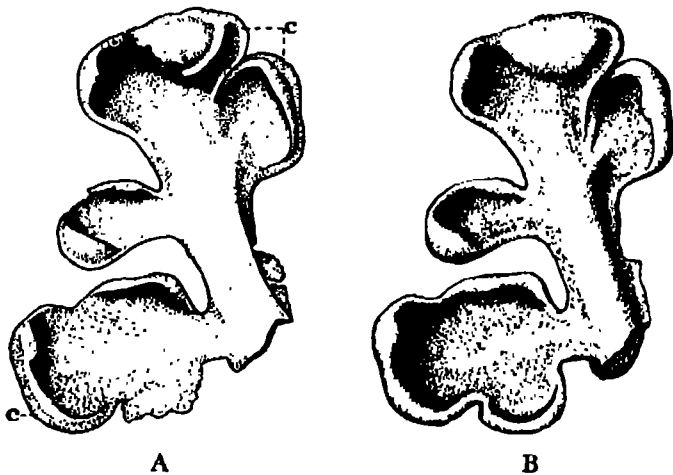


FIG. 4—A, drawing of part of a transfer preparation of *Sphenopteris nummularia* (Walton Collection 35) showing a clearly defined compression border at c, c. B, reconstruction of the same specimen before fossilization.

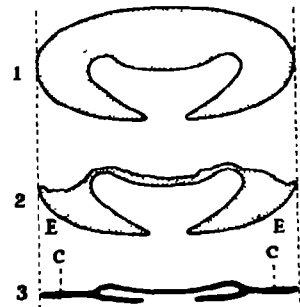


FIG. 5—Diagram to illustrate the effect of vertical compression in sediment on a leaf with inrolled margins. c, compression border.

has in his collection a transfer preparation of *Sphenopteris nummularia* (fig. 4A) in which the leaflets have in vertical section approximately the form seen in fig. 5₃. From the appearance of the specimen the leaflets undoubtedly belonged to an immature frond with inrolled leaf margins. The presence of the flat border (c, c, figs. 4 and 5) can be best explained as the result of vertical compression of a leaf such as that shown in fig. 5₁. Here, however, after the upper part of the leaf had collapsed the pressure of the overlying matrix was transmitted by the core of sediment in the hollow of the leaflet to the matrix below the centre of the leaflet. This was pressed downwards so that the matrix on either side at E and E rose relatively and compressed the leaf to the form seen in fig. 5₃.

Other examples of leaf forms modified in fossilization will be given in the descriptions of species of *Annularia* which follow.

II—DESCRIPTIONS OF THE FOLIAGE OF SOME SPECIES OF *Annularia* STERNBERG*

Although in the living genus *Equisetum* the leaf is small and ineffective as a photosynthetic organ in the Palaeozoic representatives of the Equisetales the leaves were well developed and undoubtedly represented the main photosynthetic part of the plant. Of the three more important form-genera of Palaeozoic Equisetalean plants which are based on the form of the leaf and leafy shoot, *Asterophyllites*, *Annularia*, and *Lobatannularia*, *Annularia* is the most abundant. It reaches its maximum abundance in the Upper Carboniferous where it is a familiar constituent of most Coal-Measure floras.

A large number of species of *Annularia* have been founded on the outline form of the leaves and shoots of specimens preserved in the form of compressions. The shape of the leaves and their manner of arrangement on the shoot are practically the only criteria which have been used in distinguishing one species from another. As a result some of the species are exceedingly ill defined and the nomenclature and synonymy of the species in the genus are very involved. A good deal of confusion is due to the fact that in the fossil Equisetales as in the fossil Lycopodiales the leaves on the different orders of branches on one and the same plant may show considerable differences. The leaves on the main axis or large branches of *Annularia sphenophylloides* ZENKER are quite different in size and shape from those found on the ultimate branches. The best account of the genus *Annularia* and the systematic relations of its species is given by JONGMANS (1911 and 1914, pp. 5, 235).

Of the internal structure of plants belonging to this genus practically nothing is known. CARPENTIER (1924) has described some structurally preserved leaves from the Permian of Autun or the Stephanien of Grand Croix (Loire) which he compares with an *Annularia* of the *stellata* type. HOLDEN (1935, p. 239) is, however, of the opinion that these leaves may not belong to Equisetalean plants in view of the absence of lignified bars on the stomatal guard cells. Certain authors have described as surface features certain structures which, as will be shown later, are expressions of internal structures. Our knowledge of *Annularia* falls far behind our knowledge of the genus *Asterophyllites*, the internal structure of which has been worked out in considerable detail in some species by HICK (1895, p. 179) and later by THOMAS (1911, p. 51).

Lobatannularia is only known from incrustations.

JONGMANS (1911, p. 235) describes the genus *Annularia* as follows: "Leaves linear, lanceolate, or spatulate, uninerved at the base and fused to a more or less distinct sheath. The individual leaves do not all make the same angle with the stem but lie in the same plane with the branches and the stem. The leaves of a whorl may not always be of the same size, the lateral leaves may be longer than the upper and lower ones." He points out that it is not always possible to say for certain

* A preliminary account of some of the forms described in this memoir was given at the meeting of the British Association in 1925.

that a particular species of *Annularia* represents the foliage of only one type of Calamitean stem and that the recognized species of *Annularia* are in most instances probably merely type-assemblages.

JONGMANS and other authors state that in *Asterophyllites* the leaves are attached separately at the node and that in *Annularia* they are joined to a sheath, but Dr. JONGMANS has told the writer recently that this distinction is not reliable and that it is possible that there is no sharp separation in this respect between the two genera *Annularia* and *Asterophyllites*. In some species of *Asterophyllites* the swollen node when flattened in a compression closely resembles a sheath, while in some species of *Annularia* it is impossible to distinguish a distinct sheath. The form of the leaf-mosaics found in these Palaeozoic genera has been fully discussed by HALLE (1928, p. 230) and will not be dealt with here. Recently a new genus *Carpannularia* has been proposed by ELIAS (1931, p. 116) for some American examples of the species *Annularia stellata* Schl. with which he found associated structures which he has interpreted as seeds. Some of these seed-like bodies he found attached to the specimens he describes. He raises the question as to the possibility of these structures being tubers but finally decides that they are seeds. The writer does not consider that the evidence justifies him making this decision. There is in the first place no evidence apart from their ovoid shape to support the view that they are seeds and they do not form part of a cone, as one might have expected on morphological grounds in a seed-bearing Equisetalean plant. On the other hand, they bear a very close resemblance to the tubers of *Equisetum* and they are borne in a corresponding position on the shoot. It is well known that the shoot of *Equisetum* is exceedingly easily influenced by environmental conditions; branches and roots which would not normally develop on an aerial shoot beyond the stage of a small bud at the base of a leaf sheath may be caused to develop by burying the shoot in the soil or enclosing it in a damp atmosphere. The description by ELIAS of the "seeds" as having the form of thin shell of carbonaceous matter is very reminiscent of the exhausted tubers of *Equisetum maximum* L. It is by no means inconceivable that shoots of *Annularia stellata* might have responded to burying or to the influence of a damp atmosphere by producing tubers from dormant buds at the nodes. Similar tuber-like bodies are found associated on the same slab of shale with *Annularia stellata* in a specimen in the Kidston Collection (No. 310) in the Geological Museum at South Kensington.

The investigation, of which the results are given here, was undertaken in an attempt to get more precise information about the form and structure of the leaves and shoots of *Annularia*. Some interesting and hitherto unobserved features of some of the common species have been discovered, but it has been found in addition that some of the forms that have been included under one specific name are certainly aggregates of very diverse forms.

During the course of this investigation a consideration of some of the forms assumed by leaves as a result of the factors which influence the shape of a plant fragment during fossilization led the author to make certain generalizations as to

the manner in which forces, to which plant fragments are subjected during sedimentation and fossilization, determine the shape of the resulting fossils (*see above*, p. 221).

During the last ten years the writer has had frequent opportunities of obtaining specimens of *Annularia* and has pleasure in acknowledging the friendly assistance which he has received from Professor Dr. P. KUKUK of the Westfälische Berggewerkskasse, Bochum, Dr. R. CROOKALL, and Dr. EMILY DIX. Some of the material which they have provided has been of great value in this investigation. The material which the writer has been able to obtain from various sources has been subjected to detailed examination by means of the transfer method (WALTON, 1923, p. 379) and infra-red photography (WALTON, 1935, p. 265). As the result of these investigations it has been possible to increase our knowledge of the peculiar morphological features of the leaves of this genus and to revise and improve considerably the definitions of some of the more important species.

Description of Species of Annularia

1—*Annularia sphenophylloides* ZENKER—(Figs. 8, 9, Plate 31.) The writer accepts the synonymy of this species given by JONGMANS (1914, p. 35) without criticism. It is one of the easily identified species and no one will doubt the correctness of the identification with it of the specimens described here. They are quite normal examples of the species. The ultimate branches and leaf whorls illustrated in figs. 8 and 9, Plate 31, represent portions of the main photosynthetic system of the plant. The leaves which were attached to the stems and perhaps larger branches were of a different type, lanceolate and with long points (JONGMANS and KUKUK, 1913, p. 47). The leaf-whorls on the ultimate branches consist of from 12 to 18 spatulate leaves 3 to 10 mm long and from 1 to 3 mm wide at the widest part. The apex of each leaf is rounded but bears a distinct mucro. The leaves are all arranged in the plane of the branch and thus form with it a dorsiventral shoot. In examples of this species the leaf lamina is rarely flat, it is usually convex or concave. Owing to the dorsiventral orientation of the leaves on the shoot they are all spread out in one plane and, judging from all the specimens so far examined, the convex surfaces of all the leaves on a shoot faced the same way. It is impossible to decide from the evidence whether the convex surface represents the abaxial surface or not. This concavity (or convexity) of the leaf blade must have been a very pronounced feature in the living plant for even in the fossils preserved in shale which have been subjected to considerable vertical compression the concavity is still quite pronounced.

In transfer preparations (fig. 9, Plate 31) further details may be observed. There is a distinct, narrow midrib at the base of the leaf which in the broadest part of the translucent, brownish lamina widens out to form a very characteristic expansion (fig. 9, *t*, Plate 31). This feature sometimes appears as a concavity on the convex side of the leaf on the rock and, as a result, if the leaf has been only partly freed from the matrix the apex of the leaf appears to be emarginate (*e.g.*, JONGMANS and KUKUK, 1913, fig. 7, Plate 21). ZEILLER (1888, p. 388), no doubt misled by this,

states that the leaves of this species are sometimes acuminate, sometimes emarginate. The concavity in these cases is no doubt the result of the collapse of the tissues constituting the vein expansion. The expansion of the midrib diminishes at the apex of the leaf and the vein appears to be continuous with the mucro. There is evidence that occasional simple hairs were present on the leaf, particularly round the apex (fig. 9, *h*, Plate 31). The bases of such hairs may be seen as small projections on the sides of the midrib expansion (fig. 9, *h*₂, Plate 31). Some small lighter coloured spots are present on the lamina (fig. 9, *s*, Plate 31) and it is possible that they may represent the positions of stomata. In other specimens of possibly the same species which were no doubt subjected to somewhat different conditions during decay and during fossilization, the lamina is not uniformly translucent and in transfer preparations examined by transmitted light small elongated darker coloured bodies are visible (fig. 10, *c*, Plate 31) which give the leaf a hairy appearance. It is highly probable that the "hairs" described by HALLE (1928, p. 239; Plate I, figs. 7, 8, 9) in a specimen of *Annularia sphenophylloides* are a slightly different expression of the same structural peculiarity. This hairy appearance which is seen on several species when examined even without transfer will be discussed later in relation to some other species in which the nature of these structures is more evident.

Such pronounced expansions of the vein or midrib are not found in the other species of *Annularia* which have been examined in this way. It will be noticed, however, that there is a slight expansion of the end of the vein in a specimen of *Annularia stellata* shown in fig. 29, Plate II, and in the specimen of *Annularia pseudostellata* POTONÉ figured by JONGMANS and KUKUK (1913, fig. 4, Plate 21) the presence of such an expansion is suggested by the appearance of the ends of the leaves. In living plants similar expansions are also found; they are not uncommon in the Ferns, and it is to be particularly noted that there is a distinct expansion in the strand of tracheids in the free part of the leaf in *Equisetum limosum* L. (other species of *Equisetum* have not been examined for this feature). Terminal expansions of veins are usually associated with the excretion of water in drops through special stomata. In the early morning shoots of *Equisetum* may often be found with a drop of water on the tip of each leaf. It is probable, therefore, that the expansions of the veins in *Annularia sphenophylloides* were related to hydathodic activity.

2—*Annularia galioides* LINDLEY and HUTTON sp. (fig. 11, Plate 31)—This species has a similar leaf mosaic to *A. sphenophylloides*, but there are larger gaps between the ends of the leaves owing to the more lanceolate shape of the leaf. There are as a rule about eight leaves in a whorl. In some specimens with small leaves the leaves are so broad that they are almost circular in outline. A mucro has never been observed on the rock. In transfer preparations the lamina is partly translucent and elongated darker structures are present which appear to be of a similar nature to those seen in some specimens of *A. sphenophylloides*. These darker inclusions in the lamina for the most part run parallel to the margin of the leaf or to its long axis and are most concentrated along the centre of the leaf. They converge in the tip of

the leaf and seem to form a short rather ill-defined mucro which did not apparently lie in the plane of the leaf for it does not appear in hand specimens. These observations confirm JONGMAN's suggestion that the apparent hairiness of this species might not be due to hairs but to some internal cell network (JONGMANS, 1911, p. 258).

The specimen figured here (fig. 11, Plate 31) came from approximately the same locality as Lindley and Hutton's type of the species (LINDLEY and HUTTON, 1833, Vol. I, p. 79, fig. 2, Plate 25).

3—*Annularia Jongmansii* sp. nov. (fig. 6, and figs. 12, 13, 14, 15, Plate 31)—This species is fairly common in the Upper Carboniferous of the Central Valley of Scotland. I have also seen a specimen from a boring from the Dutch coal-fields in the Kidston Collection, and Dr. JONGMANS tells me that he has found it frequently in the Dutch Limburg coal-field. He has recorded it in manuscript under the name *Annularia radiata* forma *hirsuta*. In outline form the leaves and shoots are closely similar to those of *Annularia radiata* BGT. and more especially with *Annularia fertilis* STUR and *Bechera dubia* STERNB. which most authors regard as the same species as *A. radiata* BGT. It is probable that several of the plants recorded in the literature of fossil-plants as *A. radiata* belong to *A. Jongmansii* which may have a very extended geographical distribution in the Upper Carboniferous. One other quite distinct type of leaf which from its appearance on the rock would have in the past been identified as *A. radiata* will be described later. It is clear, therefore, that *A. radiata* can now only be used as a group name for specimens



FIG. 6—*Annularia Jongmansii* sp. nov. Part of a penultimate branch bearing part of several ultimate branches. Nat. size. (Pb. 1131, Hunterian Museum, University of Glasgow.)

which have the outline form of the plant which has been called *A. radiata*.

In *Annularia Jongmansii* (fig. 6, and figs. 12, 13, 14, 15, Plate 31) the penultimate branches have inter-nodes about 20 mm long and the ultimate branches are arranged distichously. The inter-nodes on the ultimate branches are about 9 mm in length. In fig. 6 a specimen with parts of six ultimate branches is shown in outline. It is clear from the regularity of the branches and leaves that the ultimate branches and leaves were originally in one plane, the foliage forming a characteristic mosaic. The leaves vary from 4 to 10 mm in length and from 1 to 2 mm in width at the broadest part, and are arranged in whorls of from 9 to 13. Each leaf is slightly spatulate with a median midrib. A mucro is rarely seen on the stone.

In transfer preparations the leaf is usually partly translucent (figs. 12, 13, Plate 31) and the "hairy" appearance is most pronounced. The specimen shown in the figure was originally on black coaly shale and showed the "hairy" appearance on its surface even before transfer. From a study of several transfer preparations of this species it is clear that in all probability each leaf bore a distinct mucro (figs. 12, 13, Plate 31). In the relatively translucent lamina is a conspicuous system of elongated darker structures of cellular dimensions which are obviously homologous with the dark inclusions found in the lamina of *A. sphenophylloides* and *A. galioides*. It is these darker bodies in the substance of the lamina which cause the carbonaceous film of the lamina to be thicker where they are present and appear to be covered with hair-like structures. These dark bodies in the lamina have never been found projecting beyond the translucent part when the margin is complete (fig. 13, Plate 31), they are never separated by matrix from the lamina at any part of the leaf, and have not as yet been found lying loose in the matrix. If fig. 15, Plate 31 is examined, it will be seen that they have blunt ends and that they are usually grouped in series. The form and arrangement of these structures clearly suggests that they are for the most part not on the surface of the lamina but are internal. They are almost certainly to be regarded as cell inclusions. True hairs occur on the plant and they are present on the internodes (fig. 13, *h*, Plate 31) and at the nodes (fig. 14, Plate 31). All these undoubted hairs are, however, of a translucent brown colour and not like the structures in the lamina of the leaf.

Dark coloured carbonaceous cell contents of a similar shape to those found in the leaf of *Annularia Jongmansi* occur in the leaves of *Asterophyllites* found in petrifications (THOMAS, 1911, pp. 60-80). In *Asterophyllites*, according to THOMAS, these dark inclusions are found in a few of the epidermal cells, which are elongated in a direction parallel to the long axis of the leaf, in elongated cells of the mesophyll which are orientated with their axes at right angles to the long axis of the leaf, and in large numbers in the elongated cells forming the bundle sheath of the leaf. If now the specimen of *Annularia* sp., of which a transfer preparation is shown in figs. 16, 17, Plate 31, be considered it will be noticed that the distribution of dark cell contents in the flattened incrustation corresponds very closely with the arrangement found in *Asterophyllites*. In other words, if a leaf of *Asterophyllites* was vertically compressed the cells with dark contents would form a similar pattern to that seen in fig. 17, Plate 31.

The leaves in *A. Jongmansi* appear to be united to a short collar or sheath at the node (fig. 14, Plate 31). In some transfer preparations the rings of thickening of the protoxylem tracheids in the inter-nodes are visible (Walton Collection, Slide 429).

Annularia Jongmansi sp. nov.

Diagnosis of Species—Leaves spatulate, from 10 mm to 5 mm in length, and from 1.3 mm to 0.5 mm broad, mucronate. The leaves number from 9 to 13 in a whorl and lie in the same plane as the branch which bears them. The leaves in a whorl are of approximately equal length. The ultimate branches of the plant are borne distichously on the penultimate branches. The branches are indistinctly

ridged longitudinally. The inter-nodes on the ultimate branches are about 9 mm long, on the penultimate branches they are about 20 mm long. Simple hairs are present on the inter-nodes and nodes. Under certain conditions of preservation a pronounced reticulum of dark cell contents is visible in the lamina of the leaf.

Westphalian Series, Upper Carboniferous :

Western Europe

Type Specimen ; Walton Collection, Slide 423

4—*Annularia radiata* BGT. forma (figs. 16, 17, Plate 31)—The specimen, of which the transfer is shown in fig. 16, consists of an ultimate branch showing part of four leaf whorls. The leaf is lanceolate and mucronate. The reticulum of dark cell contents is very conspicuous and reference has been already made to it in the account of *A. Jongmansi*.

This plant evidently falls into the *radiata* group on the basis of the outline of the leaf. It seems to be distinct from *A. Jongmansi* in which the leaf is slightly spatulate. Westphalian Series. Scotland.

5—*Annularia fimbriata* sp. nov. (figs. 18-21, Plate 32)—It is highly probable that this species may be at a later time recognized in specimens which have been referred to *Annularia radiata* BRONGNIART and that without the preparation of a transfer it may be impossible to distinguish it from the forms usually placed in BRONGNIART's species solely on the strength of outline form. The description of this new species is based on two sets of specimens one from Lancashire given to me by the late Dr. KIDSTON as specimens of *A. radiata* BRONGNIART "vera," the other from near Bristol, given to me by Dr. CROOKALL.

When examined on the rock these specimens appeared to possess simple, nearly linear leaves with a smooth surface. Judging from the somewhat fragmentary material, there must have been about fifteen leaves in the whorl. The leaves narrow slightly at the base and taper off towards the apex. No complete leaf tips were represented in the Lancashire specimens, but in the Bristol specimens a distinct mucro is present on the complete leaves. In transfer specimens these characters are seen very distinctly (fig. 18, Plate 32), and it appears as if the leaves were joined to a narrow collar or sheath at the node (fig. 18, s). This collar might be merely the flattened cortical tissues of the slightly swollen node.

The surface of the leaf exposed by the transfer process is, however, very different from that seen on the untransferred specimen. In the transfer there is a shallow median groove about one-third the breadth of the leaf which extends from near the base up to near the apex. Attached to the two sides of the groove and extending over it are short hairs (fig. 19, r, r, Plate 32) the edges to which the hairs are attached slightly overhang the groove. The floor of the groove is slightly convex, the convexity representing the midrib of the leaf. One specimen was obtained which had been exposed by the matrix splitting over the grooved surface of the leaf. The edges of the groove with their rows of hairs were removed on one-half of the matrix and the

rest of the leaf consisting of the greater part of the lamina on the other part of the matrix. A transfer preparation from the part of the matrix with the edges of the groove was made (fig. 20, Plate 32). It shows the edges of the groove and the two rows of hairs (*rr*) and in places small fragments of the coaly lamina. Occasional hairs were present on the surface of the floor of the groove (fig. 21, *h*₁, Plate 32). All these hairs appear to be unicellular.

The simplest way to interpret these compressions is by supposing that the original leaf was gutter-shaped and had a cross-section like that suggested in fig. 5. It should be noted that in fig. 19, Plate 32, there are flat borders (*ff*) on the outer sides of the rows of hairs, the significance of this feature is discussed on p. 225.

Annularia fimbriata sp. nov.

Diagnosis of Species—Leaves linear, lanceolate, terminating distally in an acute apex which is prolonged into a distinct mucro. The edges of the leaf are inrolled and each edge has a series of short simple hairs which project over the concave surface of the leaf. Simple hairs are also found occasionally on the surface of the concave side of the leaf and at the nodes. The leaves are between 7 and 15 mm in length and from 1 to 2 mm broad and number from 15 to 20 in each whorl. The leaves lie in the same plane as the ultimate branch which bears them.

This species has so far only been recognized in specimens from the Westphalian Series in the Bristol and Lancashire coal-fields.

Type Specimen ; Walton Collection, Slide 396

6—*Annularia stellata* Schlotheim. sp. (figs. 24–29, Plate 32).

(For synonymy see JONGMANS, 1914, p. 41. *Carpannularia americana* ELIAS is also a synonym.)

Annularia stellata, judging by the figures which have been published by many authors, is a very variable species. It is probable that several of the forms are merely different types of preservation of the same species. In order to understand how such variety in form is found in fossils belonging to possibly the same species of plant the suggestions made in the first part of this paper (p. 224) should be considered.

A typical example of *A. stellata* SCHLOTH is shown in fig. 24, Plate 32. Parts of two leaf whorls are shown. The leaves are convex with a slightly sunken groove down the centre (fig. 25, Plate 32). The surface of this depression is flat and is marked with small projections (not shown in the figure) which probably represent the points of attachment of hairs (*cf.* POTONÉ, 1893, Plate XXIV, fig. 6). By cutting into the shale it was possible to see the form of the cross-section of the compression. A transfer preparation was made from three of the leaves shown in fig. 25. As one would have expected from the cross-sectional view, the surface so exposed was concave with a slight median elevation corresponding to the groove seen on the original specimen (fig. 25). In addition, there is a small projecting ridge on each side of the central elevation. The transfer preparation which was opaque and black was then photographed by transmitted, infra-red light (fig. 26, Plate 32). The infra-red

photograph shows three dark lines in the lamina; the central one is evidently a structure which is not revealed elsewhere and is undoubtedly the vascular strand of the leaf, or perhaps the xylem strand, while the lines on each side of it are the ridges to which reference has already been made. The hypothetical reconstruction of the cross-section of the leaf is shown in fig. 7, and it will be noticed that the suggestion is made that the ridges of carbonaceous material which appear on each side of the vein represent the sides of the midrib as distinct from the vein itself. An almost complete leaf apex is shown in figs. 25, 26, Plate 32, and there appears

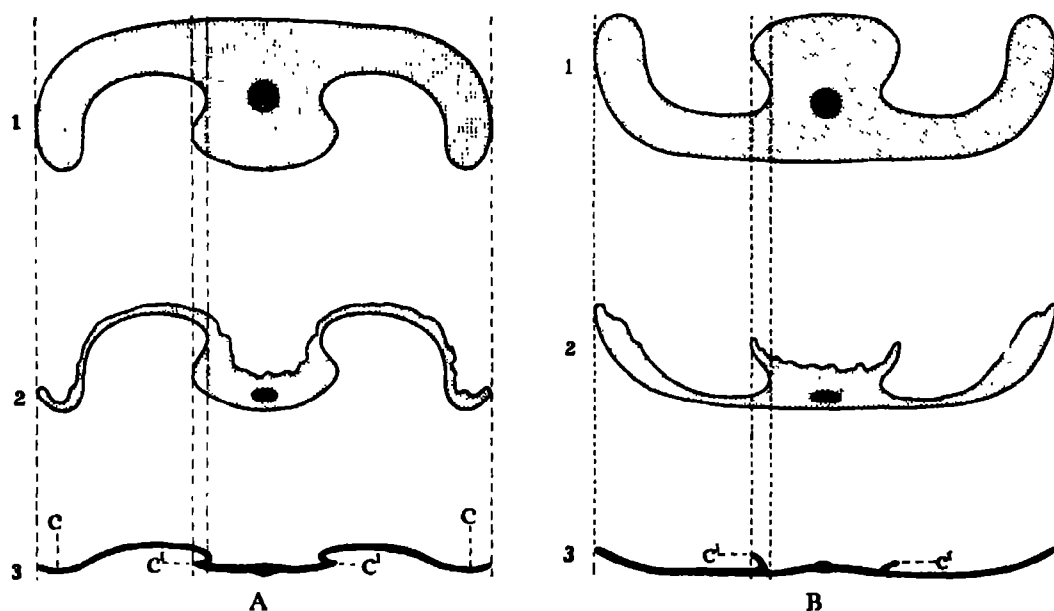


FIG. 7 (A and B)—Diagrams to represent the effect of vertical compression on a concavo convex leaf-lamina with a well-developed midrib with overhanging sides. Explanation as for fig. 3. c, compression border of leaf and c', compression borders of midrib.

to be a slight expansion of the vein and traces of a mucro. In another specimen from the Bassin du Gard, France (figs. 27, 28, Plate 32), two grooves (fig. 28, *gg*) appear on the surface of the leaf and a close comparison may be drawn between this form and that described by PORONÉ (1893, p. 162, Plate XXIV, figs. 1-6) in that there are flat borders (*säume*) on each side of the leaf (fig. 28, Plate 32). The original form of leaf which may have given rise to this type of incrustation is suggested in fig. 7A 3. The only difference between this form of *Annularia stellata* and that described above is that here there was probably a greater curvature of the lamina giving a pronounced inrolling of the edges of the leaf. The reconstruction of this form closely resembles the actual form of the petrified leaves or bracts described by CARPENTIER (1924, p. 241) which he considers belong to a species of *Annularia* closely related to *A. stellata*. Professor HOLDEN, on other grounds, is inclined to believe that these petrifications may not belong to Equisetalean plants (*see* p. 226).

A third form of *Annularia stellata* is known in which the leaf has a more or less plane surface with a single ridge down the centre representing the midrib or vein. In completely preserved specimens a distinct mucro is shown. In the transfer preparation (fig. 29, Plate 32) of a leaf of this type from Shropshire the lamina evidently contains dark coloured inclusions similar to those found in some of the other species of *Annularia*. Here, however, their rather small size and regular arrangement suggests that they may have been contained in the epidermal cells. The resemblance between these leaves and those of the plant figured by ELIAS under the name *Carpannularia americana* is most striking. There is no doubt that the "shagreened" appearance of the Shropshire leaf and the American leaves are due to the same structure. The feathery appearance of the leaves on the penultimate branches of *Carpannularia americana* (ELIAS, 1931, p. 132, Plate 13, fig. 1a, 1b) might also be due to the same type of structures which cause the "hairiness" of the leaves in other species of *Annularia*. The original form of the cross-section of this type of *Annularia stellata* is suggested in fig. 3, B.

6—*Annularia* sp. (figs. 22, 23, Plate 32)—This plant, which comes from the South Wales coal-field, differs from *Annularia stellata* in being more lanceolate in shape and in possessing a relatively long mucro (fig. 23, Plate 32). The substance of the lamina is black and anthracitic and no internal structure is visible, so that a closer comparison with the preceding forms is impossible.

SUMMARY

I—Certain peculiarities (Compression borders, etc.) in the external form of fossil plants are described and a theory is proposed which accounts for some of the forms found.

It is suggested that there may be two stages in the process of fossilization of a plant organ. (a) The plant collapses under the weight of the overlying sediment, and, losing water, forms a layer of organic material over the surface of the matrix which formed a cast of its lower surface. (b) The compressibility of the organic material approximates to that of the sediment, and the plant and matrix together may undergo further compression with the production in them of a uniform vertical deformation.

The horizontal dimensions of the plant undergo no change, but all the vertical dimensions are more or less uniformly reduced. It is shown that the fossil formed from a thick leaf embedded with its concave surface up is not necessarily similar to a fossil produced from a leaf of the same form embedded with its convex side up.

II—An account is given of the results obtained from a detailed examination of the following species of *Annularia* by the transfer method.

The use of infra-red photography was found to be of considerable value in finding out the position of the vascular tissue in one specimen.

Annularia sphenophylloides ZENK.

- „ *Galioides* LINDLEY and HUTTON sp.
- „ *Jongmansi* sp. nov.
- „ *radiata* forma.
- „ *fimbriata* sp. nov.
- „ *stellata* SCHLOTH sp.

In *Annularia sphenophylloides*, *A. galioides*, *A. Jongmansi*, *A. radiata* forma, and *A. fimbriata* the apex of the leaf has a distinct mucro. In *A. stellata* a mucro has not been demonstrated in all the forms examined. A system of dark coloured cellular inclusions has been found in the lamina of all the species investigated with the possible exception of *A. fimbriata*, although it is probable that this may be due to the condition of preservation of the specimens of *A. fimbriata* which were examined. Reasons were given for supposing that these cell inclusions correspond to the black cell inclusions found in petrified examples of *Asterophyllites*. The presence of these cell inclusions is shown to be responsible for the "hairy" appearance of various species of *Annularia*. In *A. sphenophylloides* there is a pronounced terminal expansion of the vein, a feature which is shown to a lesser degree in *A. stellata*. It is suggested that this structure might have been connected with hydathodic activity. In *A. fimbriata* the lamina of the leaf is inrolled and bears on its margins rows of short hairs. In all the species examined it has been possible to increase considerably our knowledge of their detailed structure and to show that there is very considerable range in form in the leaves of species belonging to this genus.

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DESCRIPTION OF PLATES

PLATE 31

- FIG. 8—*Annularia sphenophylloides* ZENK. Transfer on Canada balsam. There are parts of two ultimate branches shown in the photograph. $\times 2$. Tynning Pit, Radstock. Horizon: Radstockian Series, Upper Carboniferous. (WALTON Collection, Slide 403.)
- FIG. 9—*Annularia sphenophylloides* ZENK. Transfer on Canada balsam. Parts of two leaf whorls are shown. *m*, midrib of leaf; *t*, terminal expansion; *p*, mucro of leaf; *h*, hairs or hair-bases; *s*, stomata. Same locality and horizon as for fig. 8. $\times 13$. (WALTON Collection, Slide 405.)
- FIG. 10—*cf. Annularia sphenophylloides* ZENK. Transfer on balsam. Parts of two leaves are shown. Dark cell-contents are shown at *c*. Other lettering as for fig. 9. $\times 13$. Bradford Colliery, Manchester. Yorkian Series, Upper Carboniferous. (WALTON Collection, Slide 404.)
- FIG. 11—*Annularia galioides* L. and H. sp. Transfer on balsam. Parts of about a dozen whorls are shown. $\times 2$. Moncton Main Colliery, Barnsley. Horizon: Barnsley Thick Coal, Yorkian Series. (WALTON Collection, Slide 409.)
- FIG. 12—*Annularia Jongmansii* sp. nov. Transfer on balsam of two leaf whorls. Lettering as for fig. 9. $\times 3$. Devon Tower Colliery, 1 mile S.W. of Tillicoultry, Clackmannanshire. Westphalian Series. (WALTON Collection, Slide 423.)
- FIG. 13—Part of the specimen shown in fig. 12 at greater magnification. *h*, hairs on the inter-node. Undamaged margin shown at *x*. $\times 12$.
- FIG. 14—Part of the same specimen showing part of the node, the nodal sheath *s*, and hairs *h*. $\times 30$ (approx.).
- FIG. 15—Part of a leaf of the same specimen at greater magnification. Showing a series of dark, cell-contents in the lamina at *c*. $\times 60$.
- FIG. 16—*Annularia* sp. Transfer on cellulose of an ultimate branch with parts of four-leaf whorls. *p*, *p*, mucro. $\times 7$. Brucefield Colliery, Clackmannanshire. Westphalian Series. (WALTON Collection, Slide 426.)
- FIG. 17—Part of the specimen shown in fig. 15 at greater magnification. The mucro, *p*, is shown. Long, dark cell-contents oriented parallel to the axis of the leaf and shorter, dark cell-contents oriented at right angles to the axis are also shown. $\times 39$.



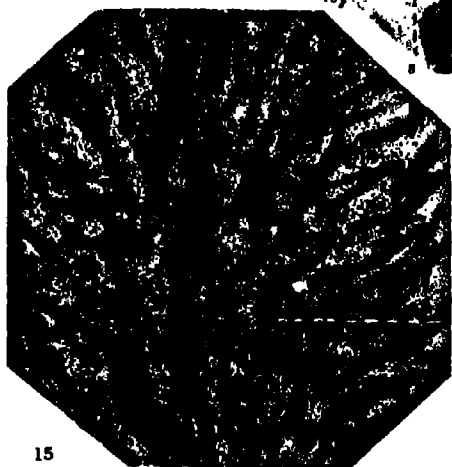
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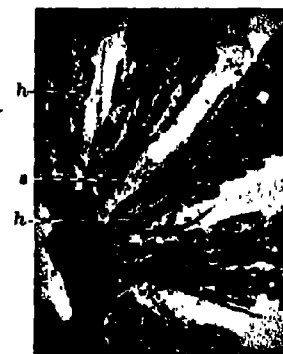
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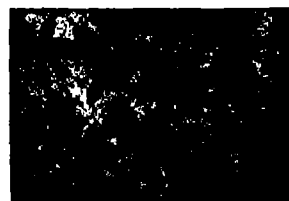
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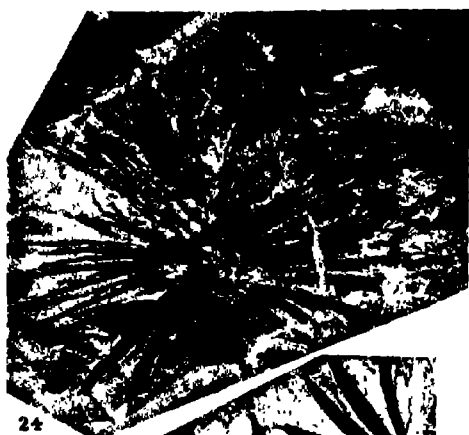
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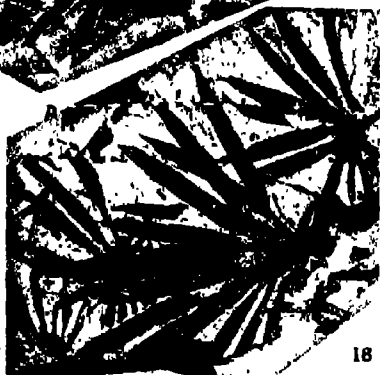
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PLATE 32

- FIG. 18—*Annularia fimbriata* sp. nov. Transfer preparation on balsam of four incomplete leaf-whorls. *p*, mucro. *s*, sheath or collar. $\times 3$. Coalpitheath Colliery, Gloucestershire. Horizon: Farrington Series. (WALTON Collection, Slide 395.)
- FIG. 19—*Annularia fimbriata* sp. nov. Transfer preparation on balsam photographed by reflected light showing parts of two leaves with inrolled margins. The two marginal rows of hairs are seen at *r* and *r* flattened borders of leaf *ff*. $\times 13$. Lea Green Colliery, $\frac{1}{2}$ mile S.S.E. from Thatto Heath Station, Lancashire. Horizon: Potato Delf Seam. Westphalian Series. (WALTON Collection, Slide 392.)
- FIG. 20—*Annularia fimbriata* sp. nov. Transfer preparation on balsam. Almost the whole of the substance of the leaf lamina has been removed on the counter part of the specimen and on this transfer only the margins of the leaves with their hairs are represented. Marginal rows of hairs most clearly visible at *r* and *r*. $\times 6$. Deep Pit, Kingswood near Bristol. (WALTON Collection, Slide 398.)
- FIG. 21—Part of the same specimen at m, fig. 13, to show the two margins of one leaf. *h*, *h*, marginal hairs. *h*, hair from surface of lamina. $\times 35$.
- FIG. 22—*Annularia cf. radiata* BOR. Transfer preparation on balsam. Nat. size. Gannoch No. 3 Colliery near Swansea, Wales. Hor. Five Feet Seam. (WALTON Collection, Slide 417.)
- FIG. 23—Part of the same specimen at greater magnification. $\times 3$. *p*, mucro.
- FIG. 24—*Annularia stellata* SCHLOTH. Leaf whorl on shale matrix. Nat. size. Camerton Colliery, Radstock, Somerset. Hor. Great Vein, Radstock Series. (Hunterian Museum, Univ. of Glasgow, No. Pb. 1132.)
- FIG. 25—Part of the same specimen. $\times 4$.
- FIG. 26—Part of the same specimen shown in fig. 24. Transfer preparation on Canada balsam of parts of three leaves, photographed on an infra-red sensitive plate using an infra-red light filter. *v*, vascular bundle of midrib. *s*, *s*, slides of midrib. $\times 4$.
- FIG. 27—*Annularia stellata* SCHLOTH. Specimen on shale matrix of one-leaf whorl. Nat. size. Fontane, Causse 4, Lower Molières Series, Bassin du Gard, France. (Miss E. DIX.)
- FIG. 28—Transfer on Canada balsam of part of the same specimen photographed by reflected light. Showing the presence of two grooves in the substance of the lamina at *gg*. $\times 3$. (WALTON Collection, Slide 402.)
- FIG. 29—*Annularia stellata* SCHLOTH. Transfer preparation on cellulose. Terminal parts of two leaves. *p*, *p*, mucro. $\times 7$. Right Bank (S.W.) of Borle Brook, 700 yds. N.E. of Billingsley Hall Farm, $\frac{1}{2}$ mile E.N.E. of the church, Billingsley, Shropshire. Horizon: Staffordian Series. (WALTON Collection, Slide 421.)



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VIII—Genetical and Taxonomic Investigations in the Genus *Oenothera*

By R. RUGGLES GATES, F.R.S. (*Professor of Botany, King's College, University of London*)

(Received 17 February, 1936)

INTRODUCTION

The present study is the result of combining genetics with taxonomy in the investigation of a polymorphic group of wild plants. It shows a degree of multi-formity which was hitherto unsuspected in the genus. All the early genetical work on *Oenothera* was done with species which had been naturalized in Europe and whose North American home was unknown. Later, DE VRIES (1913) introduced various American forms into cultivation and used them in genetic experiment, but without full taxonomic descriptions. BARTLETT was mainly concerned in describing about twenty-five new species from wild plants of eastern North America brought into cultivation, and the present writer has previously described five, all but one of them from Eastern Canada. Professional taxonomists have paid little attention to the *Onagra* section of the genus except for the occasional description of a new species from western North America, and the whole number of species now recognized and described is about 70, not counting the 17 new species and 15 new varieties described in the present paper. The reason for the neglect of the taxonomists, even after the mutation work concentrated a great deal of attention on the genus, was no doubt the difficulty that many of the characters are not well shown in ordinary herbarium material. Indeed, cultures are necessary in order to study adequately the characters of these forms; but, on the other hand, species once clearly delimited in this way can be recognized in the field, at least when well-developed plants are available, and frequently from the rosette stage alone.

The sporadic collection of seeds from widely separated localities has, however, always in my experience yielded cultures which required description as distinct new species, and this convinced me of the necessity for a much fuller investigation of the distribution of wild American forms before evolutionary conceptions could be adequately applied to the genus as a whole. It was therefore decided, when the opportunity offered, to make as full a genetic survey as possible of the *Oenotheras* to be found in a particular area. In 1932 this developed into a genetic survey of Eastern Canada. Nearly one hundred collections of seeds were made in September and October, 1932, in different parts of Nova Scotia, New Brunswick, Quebec, and Ontario, as well as sporadic collections in Wisconsin, North Dakota, and one in Pennsylvania. Other seeds have been sent to me from collections made in parts of Nova Scotia, New Brunswick, Quebec, New York State, and Saskatchewan. The various donors are mentioned under each species in the detailed description, but I wish particularly to thank Professor MARIE-VICTORIN, who accompanied me

on a seed-collecting motor journey up the south shore of the St. Lawrence from Bic to Montreal, and Mrs. WINTHROP BELL, who has sent many seeds with careful notes of their source and habitat.

From the seed collections of 1932, about 97 cultures were grown at the Courtauld Genetical Laboratory in Regent's Park in 1933. The following year some 126 cultures were grown, mainly a P_2 generation from the previous year, but partly new cultures from fresh seeds sent by Professor MARIE-VICTORIN, Professor ROY FRASER, and others. In 1935, 142 cultures were grown; these were mainly P_2 or P_3 cultures from the original collections, but a few were F_1 hybrids and a number were wild species such as *O. biennis* L., *O. nutans* ATK. and BARTL., *O. pycnocarpa* ATK. and BARTL., *O. angustissima*, *O. eriensis*, *O. novae-scotiae*, *O. ammophiloides* and *O. Victorini*. All these species came from areas being investigated, and were therefore of great value for comparison with the new cultures. Many of these cultures were, of course, recognized as belonging to species already described from these or other areas, but many more were found to belong to new species or varieties. From these cultures has gradually developed a new type of investigation, combining taxonomic with genetical methods, treating each culture from a particular wild plant as a unit, and recording the variations and mutations occurring in the cultures through three (or in certain cases two) generations. In certain instances as many as twenty-five cultures have been grown, all belonging to one species.

These cultures have furnished each year a great mass of new material for observation and description. The results are recorded below under the various species and varieties. In addition, cytological investigations have been made, mainly on the 1932 cultures, by Mr. C. E. FORD. By sampling one plant in each of 21 cultures he found in every case a ring of 14 chromosomes in the pollen mother cell nuclei. The individual cases will be recorded under the various species and are listed in Table XXVII. From this and other evidence one can conclude that throughout north-eastern North America, at least so far as the more northern latitudes are concerned, the *Oenotheras* of the *Onagra* group show complete catenation. The few exceptions to that condition thus far found among wild species in North America are as follows: In the large-flowered species *O. grandiflora* SER. from Alabama with a ring of 12, its derivative mutation, *O. lutescens*, being homozygous with 7 free pairs; and *O. Hookeri* TORR. and GRAY from the Pacific coast, California, and northwards.* The latter has 7 free pairs but its ally *O. franciscanana* BARTL. has a ring of 4. Recent work (CLELAND, 1935) indicates that the Californian forms, in strong

* In cultures of *O. rhombipetala* NUTT. from western N. Dakota, Mr. C. E. FORD has found that it has 7 free pairs of chromosomes, but in petal-shape and other characters this species stands apart from all the *Onagras*, and it belongs to the subgenus *Raimannia*. On the other hand, *O. Agari*, which is naturalized in Australia and is nearly related to *O. stricta*, belongs in *Raimannia* and frequently (SHEFFIELD, 1927) has a ring of 14 chromosomes in diakinesis. It has been shown by HEDAYETULLAH (1933) that *O. missouriensis* SIMS has seven free pairs, but this large-flowered species is a perennial with strongly winged fruits and is generally placed in a separate genus, *Megapterium*. Catenation appears, therefore, to be circumscribed in its occurrence in the Onagraceae—a speciality mainly of the subgenus *Onagra*.

contrast to the eastern ones, have not gone further than a ring of 4 in their catenation. Forms with small flowers, which are apparently derivatives from *O. Hookeri* (GATES, 1915), occur further north in British Columbia, however, and it will be important to determine whether these have increased their catenation like the small-flowered northern forms on the eastern side of the continent. It will thus be possible to decide whether, as I suspect, the chromosome ring formation increased to a maximum as the *Oenotheras* moved northwards both in the east and the West, or whether the western forms lack the power to form a ring of 14 such as the eastern forms possess.

The original seed collections were made, as a rule, from single wild plants. Occasional exceptions to this rule will be mentioned in the text. The seeds from one plant generally yield a culture which is uniform except from the occurrence of an occasional mutation. All such mutations and other derivatives or "segregates" in later generations will be described. That the seeds from a wild plant generally breed true is to be expected, since these forms with small flowers are all normally self-pollinated, and although highly heterozygous yet they remain constant owing to the chromosome catenation.

The geographical aspect of these cultures is also of great interest. Thus if seeds from different plants in the same colony or the same locality are grown, they often yield identical cultures, or the differences may be in single characters such as mean petal-length or the size of the papillae on the stem; or they may be so small that only statistical treatment would bring out a difference in certain characters. Many striking cases have been observed where two adjacent cultures, grown under as nearly identical conditions as can be obtained, show some constant visible difference of this kind which is obviously not environmental in origin. On the other hand, different cultures from the same area usually belong to the same species and frequently show no constant visible difference whatever. Not infrequently two or even more species may, however, be represented in the same area, or even in the same colony. Three years of observations of these various kinds of difference have led to conclusions regarding the nature and evolutionary value of the different types of variation in wild *Oenotheras*, which will be briefly outlined later in this paper.

From the geographic aspect also it has been of great interest to compare the cultures from adjacent areas and determine as far as possible the geographical distribution and relationships of the various forms. These forms will be described as far as possible in the order of their geographical arrangement, but the present data are sufficient to give only a preliminary conception of the distances to which particular species have spread and the possible lines of their dispersal. Certain forms appear to be coastal and others inland in their distribution. In general, in passing from one area to another, form succeeds form in the way that might be expected, depending on the topography of the country and other conditions. But very occasionally a form appears in an unexpected locality, and such cases may be due to the action of man. The *Oenotheras* nearly all prefer a disturbed sandy soil. Railway embankments furnish such conditions, and *Oenotheras* are frequently

found here. They will therefore tend to spread along railway lines, and certain cases of distribution may perhaps be explained in this way. Seeds may also be carried occasionally in hay and other crops from one part of the country to another. In the main, however, the present distribution appears to be a natural one. The disturbed soil of roadsides is a favourite haunt for *Oenothera*, and it is probable that the activities of man during the last three centuries in Eastern Canada have given opportunities for their spread which did not exist when the country was mainly forested. The clearing of forests, cultivation, and other soil disturbances by man have probably led to collisions between forms which were formerly isolated from each other. Although natural crossing is apparently unusual among these forms; yet it does occur. Owing to the catenation, such hybrids will breed true and will constitute in some cases new species or types generally intermediate between the parents and indistinguishable by any mark from the older species. The original differences must have arisen under isolation, apparently as gene mutations. From further studies it may be possible to determine how important crossing has been in connexion with the present polymorphic condition of the genus.

A genetic survey of this kind leads to a realization of the endless variety of natural forms in a genus such as *Oenothera*. However detailed it may be, the limitations of space and time make impossible a full analysis of the population of any extended area, or an exhaustive treatment of the contents of even a single colony. Many years of observation lead to the conclusion that any considerable colony of *Oenothera* contains hosts of minute scarcely observable gene differences, as well as a much smaller number of more marked and easily observable differentials. All these differences must apparently have originated at some time as gene mutations. Any strain brought into cultivation from a single plant must of necessity lose the richness of minute mutations which are present in the whole colony from which it is derived. The number of such minute mutations in the species as a whole will be very high. Some of them will be of physiological nature and subject to natural selection. These innumerable minute mutations furnish the materials which DARWIN had in mind when he wrote of continuous variations and their natural selection.

The somewhat larger mutational differences which are constantly being observed in the wild *Oenotheras* are in such features as length of petal, wide or narrow petals, red or white midribs, red or green papillae on the stem or the ovaries or the sepals, red or green teeth or glands on the leaf margins, size of papillae on the stem, and so on. There is no evidence that these characters are of any selective value whatever. They occur indiscriminately in many populations and in many species, but they, of course, belong to large linkage groups of characters in which selection of physiological characters may go on from time to time.

All other specific differences are, no doubt, also genically controlled, but in many cases several genes will be involved in a particular character, and genetic analysis in the presence of catenation can only be made after elaborate experimentation. In many such characters it is difficult to separate genic effect from fluctuation and from the effect of luxuriance or favourable conditions of growth. Thus in various

species the later rosette leaves and lower stem leaves tend to be strongly pinnatifid at base, yet this character will only be well expressed in relatively luxuriant plants and may be more or less suppressed if the plant grows under unfavourable conditions. On the other hand, some species, no matter how luxuriantly grown, will not show this character. This brings us to another point, namely, the marked effect of slight environmental differences. Plants grown near a row of trees which cut off some of the sunlight in the late afternoon are markedly smaller in the development of all their parts. While this shows that light intensity is a limiting factor in their growth, as is to be expected, yet the resulting differences have to be taken into account in drawing up the descriptions. Among wild plants, which often grow under far from optimum conditions, characters, especially of the leaves, are frequently suppressed or modified. These plants also respond strongly to environmental conditions in the later seedling or young rosette stages. In nearly all species, if the plant does not begin to form a stem with internodes by a certain date in the season, which varies from year to year, it will remain a rosette until the following year. Under other conditions stem formation may begin very early in the development and the rosette will then be omitted altogether.

The seeds for these cultures were sown each year in the greenhouse in February or early March, pricked off into boxes, later transferred to cold frames, and finally planted out about the end of May. The more strongly biennial forms even then have difficulty in forming a stem during the summer season, but many species if brought on too rapidly will, especially in a warm summer, tend to bolt, some of them practically omitting the rosette and flowering early, while others form a full rosette and only come into flower some weeks later. "Late" and "early" plants may occur in the same culture, but the condition is not inherited and is apparently a response to particular temperature or growing conditions at a certain stage of the plant's development. On the other hand, species differ markedly in the strength of their biennial habit, and all degrees occur in this respect.

Investigations of this kind under somewhat different climatic conditions from their natural habitat, necessitate continually keeping in mind the responses of the plants. In general it appears that specific characters are but little affected by the climatic differences involved. There are, however, certain marked exceptions. Thus *O. insignis* BARTL., a prairie species from Saskatoon (p. 337), grown in England, retains at first its habit of a short stem producing flowers and fruits not only from the lowest internodes but even from the rosette; but many of the plants remain rosettes and flower the following year, producing tall stems without basal flowers, i.e., they at once lose their prairie adaptation. Again *O. albinervis* from North Dakota (p. 339), where it grows under arid sandy conditions, has smooth leaves with conspicuous silky pubescence, whereas in English conditions of climate and soil the leaves are strongly crinkled and the silky pubescence fails to appear. Under these circumstances the leaves are extraordinarily like those of the mutant *O. rubrinervis*. These marked changes of habit have been observed, however, only in species brought from the relatively arid Western plains, and not in all of those. For instance, *O. rubricapitata*

(p. 343), a North Dakota species which was found growing by a pond in conditions of partial shade from trees, does not appear to be altered at all when grown in England, and the same is true of most other species.

Another category of characters which appear in *Oenothera* may be known as *evanescent characters*. Several such have been observed in these cultures. An example may be taken from *O. albinervis* (p. 343). Two strains of this species from different localities were identical in every respect except that *for a short period* one strain showed a pale red spot at the base of the petals in all the flowers, while in the other there was no trace of this spot. Another example of an evanescent character is in *O. niagarensis* (p. 326), in which, during a part of the season, there is a touch of pale red at the base of the petiole of the stem leaves. Later in the season this completely disappears, but it is always there in the early part of the season and is therefore a specific character. Another striking example is in an undescribed species from St. Jerome near Montreal. Here, throughout the main part of the season the filaments are yellow, as they are in all other species of this group. Towards the end of the season, however, with lower temperature they become brilliant orange to red. This character is uniform in all late flowers of this species and has been observed in two seasons, but no trace of it has been seen in any other species.

I am greatly indebted to the Director of Kew for help given in connexion with these experiments, to Dr. T. A. SPRAGUE, and especially to Mr. N. Y. SANDWICH, whose notes on many of the cultures were of great value in drawing up the descriptions; but I am alone responsible for delimitation of the species and varieties. Complete plant specimens from many of the cultures are now at Kew, and several other sets of specimens from the same cultures are being prepared for circulation to other leading herbaria, an original set of representative specimens being retained in the herbarium at King's College. The many photographs which are necessary for descriptive work of this kind have been carefully taken by Mr. C. S. SEMMENS.

In drawing up the descriptions of the various species, the numbers attached to the various cultures in each year, as well as the number of plants in each culture, are given in the relevant tables. Thus under *O. paralamarckiana* (Table I), culture No. 1 of 1933 contained 50 plants. Each culture of 1934 was obtained by selfing a plant of the previous generation, unless the contrary is stated. Rarely, where stated, open-pollinated seeds were used if for some reason selfed seeds were not available. In such cases the offspring were usually uniform and like the parent, showing that crossing had not taken place. Thus in the P₂ generation of *O. paralamarckiana*, grown in 1934, culture 18 contained 25 plants, culture 19 one plant, and culture 124 18 plants. In the text these cultures are referred to as 18.34, 19.34 and 124.34 respectively. Similarly in 1935 another P₂ culture of four plants was grown as 19.35, and in the same year eight P₂ cultures were grown, each from selfing a plant of the previous generation, as shown in Table I. Generally, when more than one daughter culture was grown it is because the parent plants showed some difference which required further investigation. It is to be emphasized that in every case throughout the experiments, with a few exceptions to be mentioned, each culture is derived from selfing a single plant

of the previous culture. In the pedigrees of cultures for each species, the particular culture on which the description is based is underlined. Thus for *O. paralamarckiana*, culture 19 of 1934, is underlined. In a very few cases the description is taken from two cultures, both of which are underlined.

The varying numbers of plants in different cultures depend upon the amount of germination or the space available. In 1933 50, or sometimes 100, plants were grown in each culture. Wild seeds always germinate better than those from plants in cultivation for even two years. The cause of this is unknown, except that it is due to delayed seed germination. The first flowers from a plant produce seeds which show the most marked delay in germination, but even those from the middle of the season germinate badly in comparison with wild seeds. As a single capsule usually produces over 300 seeds, good germination gives far more seedlings than can be grown, but occasional complete failures occur for no accountable reason. Seed germination is also much higher in some of these species than in others.

DESCRIPTIONS AND GENETICAL BEHAVIOUR OF THE SPECIES

Under each species a full description is given followed by a short Latin diagnosis, selecting the main characters. The photographs here published are selected from a much larger number. The relationships and any genetical peculiarities of the species are then discussed. The order of the species is, so far as possible, geographical, beginning with Massachusetts, then New York, then the numerous species and varieties recognized in Nova Scotia and the adjacent portion of New Brunswick, then up the south shore of the St. Lawrence in Quebec, then the Ontario localities around Toronto and Windsor, finally the species from Saskatchewan and North Dakota. These species are collected into Table XXVII (p. 348) to show their geographical distribution and that of their varieties. It will be obvious that even in areas where the most intensive study has been made, still more could be done before the genetic survey could be regarded as complete, while there are large intermediate areas still untouched.

O. paralamarckiana n. sp.

From seeds collected at Penzance, Woods Hole, Massachusetts, September 1932, the original plants growing tall and erect by the roadside. The following cultures have been grown. Those of 1933 and 1934 have already been briefly described

TABLE I

1933	1 (50 plants)									
1934		18 (25)	<u>19</u> (1)				124 (18)			
1935	19 (4)	25 (15)	26 (26)	29 (23)	119 (3)	30 (3)	31 (27)	32 (3)	33 (8)	

elsewhere (GATES and NANDI, 1935) where photographs of the species and five of its trisomic mutations are published in connexion with a cytological investigation of these forms.

Description—Rosette leaves dull green, *ca.* 16, narrowly elliptic or elliptic-oblancoate, apex obtuse to acute or shortly acuminate-cuspidate, 15–27 cm. \times 38–55 mm. (petiole 2.5–5 cm.) ; midrib white to pale pink above, green on lower surface ; leaf usually concave, surface markedly crinkled or bullate, but slightly less so than in *O. Lamarckiana*, undulation very conspicuous. Margin strongly repand-dentate below, repand-denticulate towards apex, teeth green or red. Indumentum on both surfaces softly \pm erect-pubescent, with rather long hairs of unequal length, midrib also \pm appressed-crispulous pubescent. There is also a second series of sub-appressed-arcuate hairs on mesophyll. Leaves often with one or two obscure liver-coloured blotches.

Stem erect, pale green with red papillae, 60–65 cm. high, hirsute with a longer and shorter series from red or green papillae, also arcuate-crispate-subappressed-pubescent ; strongly, thickly ribbed below bracts, basal branches shorter than central stem. Stem leaves elliptic, or lower elliptic-oblong, acute with red or greenish tip, concave, crinkled all over, 9–17 cm. \times 36–51 mm., margin very wavy, repand-dentate below, repand-denticulate above, teeth green, midribs white. Both surfaces with erect or suberect pubescence in two series of different length, the largest almost hirsute (fig. 1).

Ovary 8–12 \times 3 mm., strongly ribbed, densely spreading or patulous-hirsute from red or green papillae, shortly spreading glandular-pubescent and crispate-appressed-puberulous. Hypanthium 18–38 \times 3 mm., thick, rather sparsely patulous-hirsute and shortly glandular-pubescent. Bud-cone green with yellowish longitudinal stripes, 12–20 \times 6.5 mm., nearly terete. Sepal tips 2 mm., appressed, pink at tip. Petals 14–21 \times 16–22 mm., nearly truncate or rounded, not emarginate, opening out to form cup-shaped flower with narrow spaces between the cuneate bases of petals, surviving all day without wilting, tips tending to bend backwards. Filaments *ca.* 9.5 mm., anthers *ca.* 5 mm., reaching nearly to top of stigma in bud. Stigma lobes 5–6 mm., more or less spreading in anthesis, *ca.* 10 mm. above mouth of hypanthium. Ripening fruits green, short and stout, *ca.* 20 \times 7 mm., with a fine pubescence of long and short hairs (no red papillae).

Diagnosis—Folia radicalia surda viridia, elliptico-oblancoolata, costa alba aut pallida rubicunda, superficies manifeste bullata, undulata ; caulis erectus tuberculatis rubris aut viridibus. Folia caulina elliptica, aut inferiora elliptico-oblonga, acuta, concava, bullata, costae albae. Petala 14–21 mm. longa, fere truncata, non intra diem marescentia, stigma circa 10 mm. supra hypanthium.

This species is essentially like a *Lamarckiana* with small flowers, but the leaves are more lanceolate and pointed at the tip than in *Lamarckiana*. The 1933 culture was somewhat shaded by tall trees, and as a result the plants were smaller in size. Plants

grown in full sunlight in 1934 and 1935 were markedly more luxuriant. They show strong resemblance in foliage characters to the well-known specimen in the Jardin des Plantes which DE VRIES identified with *O. Lamarckiana*. This specimen is figured by DE VRIES (1914, Plate 19) and also by DAVIS (1927, Plate 5) and was obtained somewhere in eastern North America by MICHAUX in the eighteenth century. The luxuriance of MICHAUX's specimen indicates that it was perhaps a garden specimen from seeds collected by him. The foliage of *O. paralamarckiana* is very similar to



FIG. 1---*O. paralamarckiana*, culture 19.35.

that of the specimen in question, and in fact agrees with it in leaf shape more closely than does the *Lamarckiana* of DE VRIES because of the more pointed leaves of *O. paralamarckiana*, but the MICHAUX specimen has long petioles. There is, however, a great difference between *O. Lamarckiana* and *O. paralamarckiana* in flower-size, which almost certainly involves more than one factor for petal-length. Investigations of its trisomic mutations, which are much more frequent than in *O. Lamarckiana*, are being continued. Mr. C. E. FORD determined the chromosome catenation as a ring of 14, from a normal plant in culture 1.33.

O. pycnocarpa ATK. and BARTL. varieties

From seeds collected by Dr. G. L. STEBBIN, jr., in September, 1932, at three neighbouring localities in New York State. They were at first regarded as belonging to an undescribed species, some strains of which are strictly cleistogamic while others are chasmogamic, and still others intermediate in this character. Marked variations in several other features are found, and several of these may be due to single genes. The following 25 cultures have been studied :

TABLE II

	Hamilton, N.Y.			Clinton, N.Y.		Hamilton, N.Y.	Georgetown, N.Y.
	90 (22 plants)	91 (50)	92 (50)	93 (50)	94 (44)	95 (50)	96 (48)
1933							
1934	113 (6)	114 (11)	115 (39)	116 (12)	117 (9)	118 (27)	119 (20)
1935	108 (12)	109 (34)	110 (7)	111 (11)	112 (4)	113 (17)	114 (5)
						115 (4)	116 (20)

The plant from which culture 90.33 was derived grew on gravelly soil in a railway yard, the plant for culture 91.33 in loamy soil on the border of a field, that for culture 96.33 on a shady bank. These are all characteristic habitats for *Oenothera*, though sandy soils appear to be generally preferred. The plants and their descendants all clearly belong to one species, but the cultures differ markedly in such features as (1) flower size, (2) cleistogamy or chasmogamy, (3) red or green midribs, (4) upper rosette leaves and lowest stem leaves strongly pinnatifid or not.

Although these cultures are now regarded as all belonging to *O. pycnocarpa* ATK. and BARTL., yet it appears desirable to give a detailed description of the type culture (113.34) for comparative purposes, on account of the striking range of variations they show in different strains.

Description—Rosette leaves dull greyish-green, number of rosette leaves *ca.* 11, narrowly elliptic or elliptic-oblongate, apex acute or shortly acuminate, reaching 18–22 cm. × 35–49 mm. (petiole 4–5 cm.), flattish or slightly concave, crinkling none or slight, usually conspicuously undulate, margin repand-dentate to sub-pinnatifid below, repand-denticulate above, teeth green, midrib pale pinkish-mauve, surface finely erect-pubescent on both surfaces and crispulous appressed-pubescent on midrib (fig. 2).

Stem erect, *ca.* 108–112 cm., basal branches decumbent at base then widely arcuate-ascending, long, but shorter than central stem, which is scarcely ribbed, except very thinly so in upper part, green or tinged brownish- or purplish-red, rather sparsely patulous-hirsute with reddish papillae and rather sparsely arcuate sub-appressed-pubescent. Stem leaves ± deflexed or patulous-deflexed, lanceolate to elliptic-lanceolate, flattish, not wavy or crinkled, 15–17 cm. × 40 mm., margin sparingly repand-dentate near base, repand-denticulate above, teeth green, both surfaces suberect-pubescent, midrib pinkish-mauve or nearly white, on lower leaves

quite white, \pm appressed-crisped-puberulous above, sparsely suberect-pilose and fairly densely subappressed-pubescent below. Lower bracts \pm spreading, concave, wavy at base, lanceolate, 8-14 cm. \times 30-40 mm., tips upcurved. Upper bracts \pm spreading with upcurved tips, very concave, wavy in lower half, *ca.* 12-30 mm. long.

Apex of inflorescence very narrow, \pm flat, overtopped by higher developed buds and flowers, spike not dense. Ovary 8-11 \times 2.5 mm., fairly densely patulous- or

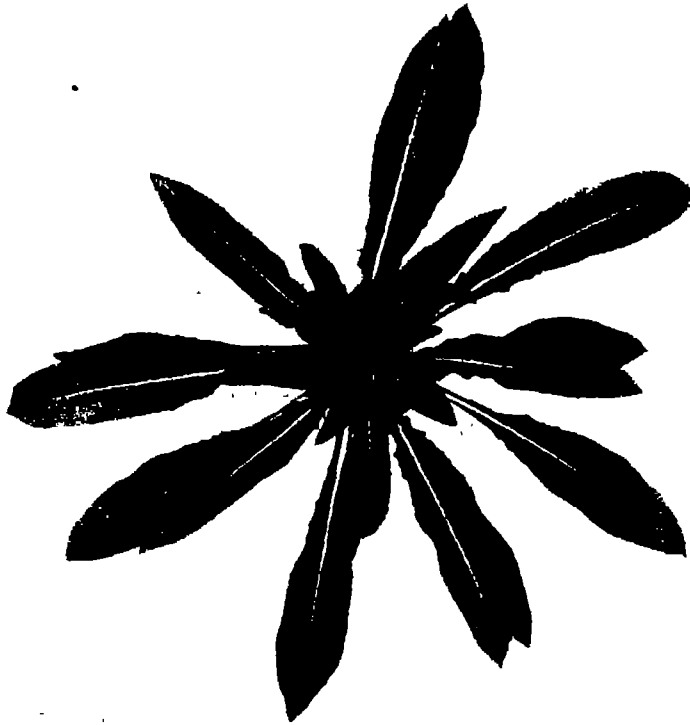


FIG. 2—*O. pycnocarpa* var. *parviflora*. Rosette, culture 115.34.

spreading-hirsute from red papillae, and spreading glandular-pubescent. Hypanthium 25-28 \times 2 mm., sparsely patulous-hirsute from white base and sparsely glandular-pubescent. Bud-cone greenish-yellow, \pm subcylindric, 9-10 \times 5-55 mm., indumentum as on ovary, but papillae white. Sepal tips 2-3 mm., somewhat divergent, reddish in upper quarter. Petals *ca.* 9-13 \times 10-13 mm., truncate or with shallow sinus, opening to 45°, not contiguous. Filaments *ca.* 7-8 mm., anthers *ca.* 4.5 mm., reaching nearly to top of stigma in bud, stigma lobes *ca.* 3 mm. long, divergent, base of stigma *ca.* 7 mm. above hypanthium. Inflorescence elongated, fruits 31 \times 7 mm., green with short pubescence and few long hairs.

In the 1933 cultures particularly there was a very marked distinction between "early" and "late" plants. Early ones formed no persistent rosette but became

bushy and flowered early, having curled bracts. Late plants formed a rosette of large leaves, came into flower much later, and had larger leaves and bracts. In some cultures these two types could be scored easily, as shown in Table III; in others this was difficult or impossible owing to the presence of intermediates. In one culture the plants were all late and in another they were all early. This difference appears to be a direct response to the conditions of culture, and shows no signs of being inherited in later generations, except in cultures 116.34 and 117.34. These were derived respectively from selfing a late and an early plant in culture 92.33. The plants in 116.34 were all mainly cleistogamic; those of 117.34 were earlier in development and were all chasmogamic. In the following generation the latter difference was completely maintained, but there was no difference in the rate of development.

TABLE III

Culture	Early	Late	Intermediate	
90.33	9	13	0	distinct (scored)
91.33	0	50	0	—
92.33	6	44	0	distinct
93.33	50	0	0	—
94.33	6	29	9?	not distinct
95.33	not classified			
96.33	15	33	0	very distinct

Other variations in these cultures may now be considered. Culture 90.33 was uniform, and cultures 113.34 and 114.34, each derived by selfing a different plant of this culture, were alike, as were their descendants 108.35 and 109.35. These cultures differed from the original *O. pycnocarpa* from Ithaca, N.Y., grown in the Gardens at the same time, in the following points: (1) plants smaller, (2) flowers smaller (petals 11 × 12 mm.), (3) red papillae on stem. This Hamilton, N.Y., strain may therefore be known as *O. pycnocarpa* var. *parviflora*, n. var.

Diagnosis—A specie differt planta minore, floribus minoribus (petalis 10–12 mm. longis), tuberculis rubris mediocris mensurae in caule.

Culture 91.33, from the same locality, differed from the above in (1) white midribs and narrower stem-leaves (35 mm. against 42 mm.), (2) slightly larger flowers (petals 12–15 mm. against 9–11 mm.), (3) appressed sepal tips. These differences remained constant in the 1935 cultures, and it was observed that the F_2 descendants of 91.33 differed in addition in (4) having no red papillae on the stems and (5) leaves longer and \pm crinkled.

The three cultures from Clinton, N.Y., showed marked differences. In culture 92.33, of the 44 (late) plants which produced rosettes, 33 had pink and 1 white midribs. The flowers were larger (petals 18–19 mm.), and all plants had open (chasmogamic) flowers. Two plants (I.2 and II.2) were selfed to produce cultures 116.34 and 117.34. No. I.2 had pink midribs and belonged to the late type. Its offspring were uniform, with rosettes, pink midribs, and some flowers cleistogamic

on each plant. No. II.2 was early. Its offspring differed from those of I.2 in that all the flowers were chasmogamous. The Clinton, N.Y., plants may be called var. *cleistogama* n. var. (fig. 3), since the three strains from this locality all showed the condition to some extent in the original or in descendant cultures. The condition is somewhat variable even in the same plants at different times, depending probably on temperature and other factors. The cleistogamic condition is caused by a failure of



FIG. 3—*O. pycnocarpa* var. *cleistogama*, in flower, culture 118.34.

the growth force at the base of the petals, which normally forces the sepals apart and so opens the flower. This may be completely absent or may be only sufficient to force the sepals apart at their base. Occasionally the sepals are burst apart or even turned back, but the petals do not unroll or separate. Flowers which never open are self-pollinated and produce seeds exactly like chasmogamous flowers; but the buds droop, turn yellow, fade, and finally fall off after fertilization without opening. Notwithstanding the variability of the condition, it is clearly inherited and probably is a

simple Mendelian difference. There is insufficient evidence as to which is dominant, but probably cleistogamy is recessive, and it is probable that it has arisen from the type through a single mutation.

Diagnosis—A specie differt floribus cleistogamis, petalis grandioribus (*circa* 15–18 mm. longis), costis albis vel rubris, foliis caulinis inferioribus pinnatifidis, tuberculis rubris in caule valde magnis.

Cultures 92.33 and 93.33 differed in that no cleistogamic flowers were observed in the former, while nearly all the flowers were cleistogamic in the latter. As already mentioned, the R and r factors (red and white midribs) were present in different plants of 92.33, and some plants evidently contained the factor for cleistogamy, while others did not, for of the two daughter cultures, in 116.34 all the plants had some cleistogamic and some chasmogamic flowers, while in 117.34 all the flowers opened properly. Culture 93.33 seldom opened a flower, and the same was true of its two descendant cultures in 1934. The F₂ cultures in 1935 were similar but with some variation. Thus 113.35, when beginning to flower on 27 July, had 105 cleistogamic and 39 chasmogamic flowers (21 of the latter on side branches), while on 5 August the flowers were nearly all cleistogamic and there were only 6 open flowers in the whole culture. In 114.35, on the other hand, all flowers were cleistogamic except some on the side branches. As shown elsewhere, (GATES 1932), the petals on side branches are always 2–3 mm. shorter than on the main stem, and this may be why such flowers open more easily. The results as regards cleistanthy are summarized in Table IV. In culture 94.33 some plants produced a mixture of open and closed flowers, some had only a few closed flowers, while in 18 plants the flowers were nearly or quite all open, and in the remainder the flowers were mostly closed. Hence while they could not all be scored, some plants were chasmogamic but the majority were cleistogamic. From the table it will be seen that cleistogamic plants produced only cleistogamic offspring, with some environmentally produced variation in the expression of the character, while pure chasmogamic plants also bred true, but there remains some doubt about the exact behaviour of heterozygous plants.

TABLE IV

O. pycnocarpa var. *cleistogama* from Clinton, N.Y.

1933	92 chasmogamic		93 cleistogamic	94 some plants cleisto.
1934	116 ± cleistogamic	117 chasmo.	118 cleistogamic	119 cleistogamic
1935	111 cleistogamic	112 chasmo.	113 ± cleisto.	114 cleistogamic

Culture 95.33, also from Hamilton, N.Y., agreed with the other cultures from that locality in having chasmogamous flowers, but this strain differed in having larger flowers, the petals being 20 mm. long (22 mm. in the descendant culture 121.34 and 18–22 × 20–30 mm. in 115.35). It therefore agrees with the type of the species

but has flowers of maximum size. The strain has therefore lost a dominant factor (or perhaps two or more linked factors) which has the effect of subtracting *ca.* 10 mm. from the petal length. Several times it has been found that strains of a species occupying adjacent areas differ only or mainly in flower size. That the difference is genetic has been shown by growing them side by side for successive generations when, as in the present case, the difference remains constant.

Culture 96.33, from Georgetown, N.Y., belonged to var. *parviflora* (petals 10 mm.) but differed from the others in the presence of several marked subpinnatifid lobes and jags at the base of the last rosette leaves (fig. 4) and the lowest stem leaves,



FIG. 4—*O. pycnocarpa*, rosette, culture 116.35.

a character of the species at Ithaca, N.Y. (BARTLETT, 1913, ATKINSON, 1918). Many of the rosette leaves were also conspicuously margined with a yellowish colour, the midribs were white, and in culture 116.35 it was noted that there were no red papillae on the stem. These features were perpetuated in culture 123.34 (petals 9 mm.) and 116.35 (petals 11 mm.). They were chasmogamic.

Summarizing this series of 25 cultures in three generations, all belonging to *O. pycnocarpa*, as observed especially in the nine cultures of 1935 (see Table II, p. 248); cultures 108 and 109 were identical, representing var. *parviflora* and differing from the type in (1) smaller flowers (petals 10–12 mm.), (2) red papillae of medium size on stem, (3) smaller plants. Culture 110, also from Hamilton, differed from 108 and 109 in

having (1) somewhat larger flowers (petals 13-14 mm.), (2) no red papillae on stem, (3) white midribs, (4) leaves longer and \pm crinkled. Culture 115, from the same locality, agreed with the previous in being chasmogamic, but differed in having (1) much longer petals (18-22 mm.), (2) no red papillae on the stem or very small ones, (3) pink midribs. Of the Clinton cultures, 111 differed from 108 and 109 in having (1) the lower stem leaves pinnatifid, (2) very large red papillae, (3) leaves slightly darker green, (4) cleistogamic, (5) petals 15×16 mm. Culture 113 was also cleistogamic, differing from 111 only in having (1) lower leaves more pinnatifid, (2) midribs pink (as in 108), (3) leaves lighter green and somewhat broader (as in 108). Culture 112 (also from Clinton) was chasmogamic and differed from 108 and 109 in having (1) larger flowers (petals 19 mm.), (2) large red papillae, (3) leaves somewhat lighter green. Culture 114, from Clinton, was essentially the same as 111 and 113, being cleistogamic and having petals 16-18 mm. long. The Georgetown culture, 116, differed from the Clinton cultures 111, 113, 114 in having (1) no red papillae on the stem, (2) midribs white, (3) many leaves having yellowish coloured edges, (4) flowers smaller (petals 11×10 mm.), (5) chasmogamic.

It will thus be seen that some of the original cultures showed segregation for certain characters in their offspring, but this was probably due to mixed pollination of certain wild plants from which the seeds for the 1933 cultures were obtained. The characters involved, each of which except flower-size is probably due to a single pair of genes, are (1) pink or white midribs, (2) lower leaves pinnatifid at base, (3) presence or absence of red in the papillae on stem, (4) large or small red papillae, (5) leaves lighter or darker green, (6) cleistogamy or chasmogamy, (7) flowers larger or smaller. Differences in flower-size were only noted when they could be readily seen by observing adjacent rows of plants. One or two measurements of typical flowers from the main stem were then made. On this basis the flower-size of the various strains can be classified as follows :

Flowers larger	Cultures 112, 115	Petals 18-22 mm.
„ intermediate	„ 111, 113, 114	„ 15-18 mm.
„ smaller	„ 110	„ 13-14 mm.
„ smallest	„ 108, 109, 116	„ 10-12 mm.

Cleistogamy has already been described in *O. cleistantha* SH. and BART. (BARTLETT, 1915) from Huntington, Long Island, N.Y., but that species is very different, having cruciate petals, very leafy and dense branching, also long hairs on the calyx and around the top of the hypanthium. Cleistogamy has therefore probably originated as an independent mutation in *O. pycnocarpa*. *O. stenomeris* BARTL., from Maryland, also has flowers which are both cruciate and cleistogamic. DE VRIES (1913) has also figured a new (undescribed) species with small flowers (p. 34, fig. 9) from Manhattan, Kansas, which he collected in 1904 and in which the flowers, as a rule, do not open. BOEDIJN (1924) describes *O. disjuncta*, another cleistogamic species collected by DE VRIES in 1904 at North Town Junction, near Minneapolis. *O. Bauri* BOEDIJN, collected by BAUR at FRIEDRICHSHAGEN near Berlin in 1918, rarely opens a

flower. This species is presumably related to *O. biennis* L. It therefore appears that cleistogamy has arisen at least five times in *Oenothera* through independent mutations—a striking instance of parallel mutations; but the relationship of *O. stenomeris* and *O. cleistantha* needs closer examination. It is also significant that in the Onagraceous genus *Boisduvalia*, *B. cleistogama* CURRAN, from California, never opens its earlier flowers although the later ones expand.

O. novae-scotiae GATES

This species, first described from near Middleton, Annapolis Co., Nova Scotia, in 1916, has been collected from a number of localities, and a large number of cultures belonging to this species has been grown. They show characteristic minor differences which will be briefly described. The native home of this species is the Annapolis Valley. A strain from Charny, Quebec, resembles this species, but its characters have not yet been fully elucidated; another strain, from Cap Tourmente, Quebec, is in the same condition. The thirteen cultures shown in Table V have been studied.

TABLE V

	Middleton, N.S.		Ruggles Road, N.S.	Kentville, N.S.
1933	4 (50 plants)	8 (50)	6 (48)	9 (50)
1934	22 (22)	27 (9)	24 (12)	25 (18)
1935	51 (32)		52 (5)	53 (26)

As the original description (GATES, 1916) was less complete, it is desirable to give a full account for purposes of comparison.

Description—Rosette leaves dull green, *ca.* 12–21, narrowly elliptic or oblanceolate, apex acute or shortly acutely acuminate, reaching 17–34 × 45–50 mm. (petiole 3–6 cm.). Undulation usually conspicuous, crinkling more or less marked, especially towards base of developing leaves, margin subentire, repand-dentate or pinnatifid at base, repand-denticulate above; midrib pink, shortly subappressed-crispate-pubescent with some suberect hairs, mesophyll suberect-arcuate-pubescent, with more erect hairs on lower surface (fig. 5).

Stem erect, 60–75 cm., numerous basal branches, decumbent at base, then widely arcuate-spreading-ascending, as long as or somewhat longer than central stem. Cauline branches numerous, ascending, flowering. Stem broadly ribbed, green or red, patulous-hirsute from red papillae and subappressed-crispate-puberulous. Stem leaves arcuate-spreading or arcuate-deflexed, slightly concave, lower elliptic-lanceolate, 13–17 cm. × 30–35 mm., acute with brownish-red tip, upper lanceolate, margin repand-dentate below, repand-denticulate above, teeth green; midrib pink in lower half; upper surface arcuate-suberect-pubescent, and midrib also minutely appressed-crispulous-puberulous, on lower surface hairs somewhat longer and more conspicuous. Inflorescence 26 cm. long, spike dense, many flowered;

lower bracts lanceolate, spreading, \pm concave, 9–11 cm. \times 30–35 mm.; upper bracts spreading 18–32 \times 5–8 mm. Apex of inflorescence somewhat depressed, about 2 cm. diameter, outer buds overtopping central, not comose.

Ovary 11–13 mm., 2.5–2.7 mm. diameter, ascending-hirsute with simple hairs from red papillae and spreading-pubescent with short gland-tipped hairs. Hypanthium 30–32 \times 2.5 mm., indumentum as ovary but sparse and longer hairs spreading or patulous. Bud-cone yellow, subcylindrical, 14–15 \times 6 mm., with



FIG. 5—*O. novae-scotiae*, rosette, culture 51.35.

long patulous hairs from white papillae and sparse, short spreading gland-tipped hairs. Sepal tips 4–5 mm., erect, appressed and parallel or slightly divergent at apex, green and yellow, pinkish at apex. Petals 18–23 \times 20–30 mm.,* opening to *ca.* 60°, much overlapping, truncate or toothed, very obscurely, widely and shallowly emarginate. Filaments 10 mm., anthers *ca.* 7 mm., overtopping stigmas by 2–3 mm. Stigma lobes 4–5 mm., 7–8 mm. above hypanthium, widely divaricating in anthesis, and protruding from flower buds towards end of season. Fruits green, 18 \times 5 mm., tapered only at apex, no long hairs nor papillae, few short hairs (fig. 6).

* In the next generation (culture 51.35) the petals measured 25 \times 29 mm.

Culture 8.33 was derived from a wild plant having distinctly larger flowers and the habit of later flowering on the cauline branches after all the fruits of the main stem were shedding their seeds. These features were equally marked in the offspring (fig. 7), the petals being $32-34 \times 32$ mm., ovary 15 mm., hypanthium 35×3 mm., fruits 20×6 mm. The catenation of this large-flowered strain was determined by



FIG. 6—*O. novae-scotiae*, in flower, culture 22.34.

Mr. C. E. FORD as a ring of 14. The type of the species had previously been shown to have a ring of 14 (SHEFFIELD, 1927). In the next generation, culture 27.34, the petals were shorter though still exceptionally wide (22×29 mm.),* hypanthium

* As this was the first flower to open on the plant, and as first flowers are sometimes abnormally small, like pullets' eggs, it is probable that the mean size of flower in this culture was considerably larger, but no further measurements were taken.

30 × 3 mm., and ovary 12 × 3.5 mm. Whether a single gene is responsible for this difference in flower-size of the two strains is not yet known. The complexes of *O. novae-scotiae* were named *grandiflorens* and *parviflorens*, the smaller petal being dominant, but this difference in petal-length did not always appear in crosses with homozygous forms (GATES and CATCHESIDE, 1932). Forms with equally large



FIG. 7—*O. novae-scotiae*, strain with large flowers, culture 8.33.

flowers have since been observed in other areas adjacent to the Annapolis Valley. It appears probable that this large-flowered form is homozygous for one or more longer-petal genes for which the type of the species is heterozygous.

Culture 6.33 was derived from seeds of several plants in an orchard on the Ruggles Road, some two miles from Wilmot and about the same distance from Middleton, on 6 September, 1932. The plants were very numerous in this orchard, where

they were a serious weed. Some plants were noted as having crinkled stem leaves alternating with smooth ones. This culture belonged to *O. novae-scotiae*, but yielded two slightly different types which could be scored as rosettes. A selfed plant of each type yielded cultures 24 and 25 respectively in 1934, in which the constant differences shown in Table VI were observed.

TABLE VI

	Culture 24.34	Culture 25.34
Rosette leaves, 1933 . . .	23 cm. \times 58 mm.	24 cm. \times 42 mm.
Rosette leaves, 1934 . . .	44 cm. \times 82 mm.	38 cm. \times 60 mm.
Midribs	White or light pink	Red
Middle stem-leaf, 1934 . .	19-20 cm. \times 43-49 mm.	20 cm. \times 38 mm.
Stem	Green, no red papillae	Red, with red papillae
Ovary	14-16 \times 3.5 mm.	16 \times 3.5 mm.
Hypanthium	28-38 \times 3 mm.	30-34 \times 3 mm.
Bud cone	18 \times 6 mm.	20 \times 7 mm.
Sepal tips	7 mm.	9 mm.
Petals	20-22 \times 26-27 mm.	26 \times 34 mm.

From the observations and measurements it was clear that Type I had wider rosette and stem leaves, less red on the midribs, and smaller flowers. The sepal tips were long and strongly appressed as usual in *O. novae-scotiae*, in contrast to *O. comosa*, *O. intermedia*, and *O. Hazelae* var. *parviflora* (*vide infra*) in which they are more or less markedly subterminal. Unlike the type of *O. novae-scotiae*, both these cultures had green teeth on the leaf margins. The broad petals were exceptionally firm, usually remaining erect on the day following anthesis. Each petal generally opened out flat and erect, the petals being folded around each other to make a cup-shaped flower.

Culture 9.33, from near Kentville, King's Co., N.S., represents a markedly distinct variety of *O. novae-scotiae* which may be called var. *serratifolia* n. var. It agrees with the species in such features as pink midribs, erect stem with red papillae, and in habit and general flower characters. But it differs in (1) narrower rosette leaves (20-21 cm. \times 32-34 mm.) tending to be acuminate and crinkled when young (fig. 8), (2) stem leaves markedly repand-dentate, (3) flowers much smaller (petals 10-11 \times 12 mm.), sepal tips short (2-3 mm.), (5) inflorescence short and compact (fig. 9). In the F_2 of this strain the seeds failed to germinate and it has not been grown since, but its distinctness is clear.

Diagnosis—A specie sic differt : folia radicalia angustiora, folia caulina admodum repando-dentata, flores multo minores (petala 10-11 mm. longa), apices sepalorum breviores, inflorescentia brevis et compacta.

O. novae-scotiae var. *distantifolia* n. var.

Seeds of this variety were collected at Newport, Hants Co., Nova Scotia, on 27 September, 1932, the plants being noted as tall, with small flowers. The following cultures were grown :—

1933	10 (50 plants)
1934	29 (5)
1935	55 (4)

Description—Rosette leaves rather dull green, lustrous along midribs, reaching 19–25 cm. × 41–62 mm., oblanceolate, apex acute, obtuse or shortly broadly



FIG. 8—*O. novae-scotiae* var. *serratifolia*, rosette, culture 9.33.

acuminate, flattish, crinkling usually conspicuous near midrib, margin repand-dentate (strongly) below, finely repand-denticulate above, teeth green, midrib white to pale pink, both surfaces regularly, not densely, subappressed-pubescent (fig. 10).

Stem erect, *ca.* 66 cm., several basal branches decumbent then widely ascending, numerous short ascending cauline branches ; stem ribbed except above, green with reddish tinge, rather sparsely patulous and arcuate-ascending hispid in two series of different length, with conspicuous red papillae and arcuate-subappressed-puberulous, branches deep red in upper half. Stem leaves arcuate-deflexed, narrowly elliptic-lanceolate, 10–17.5 cm. × 17–34 mm., margin conspicuously wavy, conspicuously

repand-dentate below, obscurely repand-denticulate above, teeth green, midrib pinkish, both surfaces suberect-pubescent, midrib appressed crisped-puberulous above, with longer \pm erect hairs below. Inflorescence lax, 21.5 cm. long. Lower bracts arcuate-spreading to deflexed, lanceolate, 4.5-6 cm. \times 8-17 mm. Upper

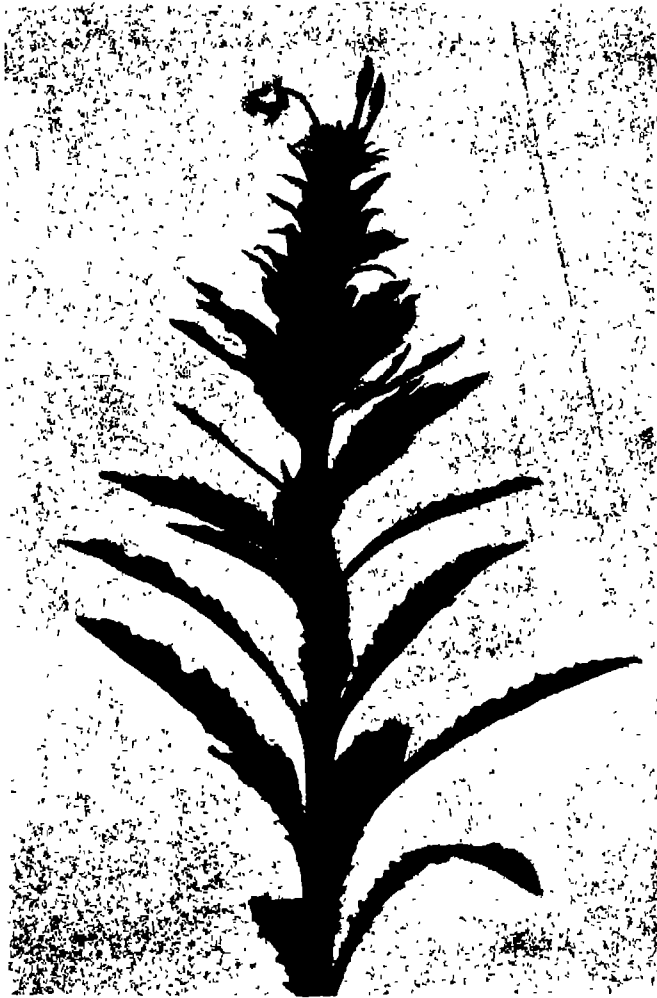


FIG. 9—*O. novae-scotiae* var. *serratifolia*, in flower, culture 9.33.

bracts spreading with upturned tips, ca. 15-20 mm. long. Apex of inflorescence overtopping highest developed buds and flowers (fig. 11).

Ovary 13×3 mm., sparsely patulous-hispid with dark red papillae and densely spreading glandular-pubescent. Hypanthium $29-31 \times 2$ mm., indumentum as ovary but sparse and papillae green. Bud-cone greenish-yellow, subcylindric, \pm 4-angled, 14×5.7 mm., indumentum as ovary but sparser, from green papillae or with reddish tinge, sepal tips 2-3.5 mm. long, reddish in upper third, appressed

or somewhat divergent. Petals opening to *ca.* 60 deg., widely overlapping, 19 × 19 mm., usually rather widely deeply emarginate. Filaments 10–11 mm., anthers 7–8 mm., overtopping stigmas by at least 2 mm. Stigma lobes 5 mm. long, divergent, 12–13 mm. above hypanthium. Fruits green, slender, 23 × 4 mm., bearing dense short hairs and sparse long ones.

Diagnosis—A specie sic differt : longa spatia inter nodos, folia angusta, apices sepalorum breves.

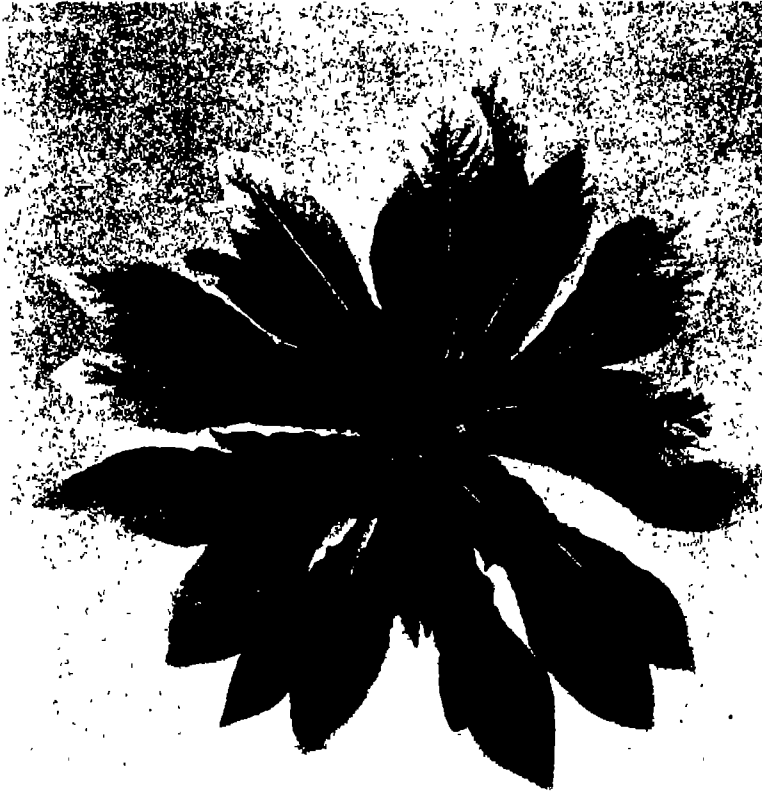


FIG. 10—*O. novae-scotiae* var. *distantifolia*, rosette, culture 29.34.

This variety differs from the type mainly in having (1) long internodes, (2) narrow leaves. In the narrow leaves and short sepal tips it agrees with var. *serratifolia*, but the latter differs markedly in the repand-dentate margins of the stem leaves and in the much smaller flowers.

O. comosa n. sp.

This species is described from seeds collected at Wilmot, N.S., in an apple orchard about two miles from Middleton, on 6 September, 1932. The following two

cultures were grown and yielded a uniform strain with numerous distinctions from *O. novae-scotiae*. Its relationships will be discussed later.

1933 5 (50 plants)

1934 23 (32)

Description—Rosette leaves dark green, narrowly elliptic, acute, reaching 23 cm.



FIG. 11—*O. novae-scotiae* var. *distantifolia*, in flower, culture 29.34.

× 39 mm. total length, smooth or nearly so ; midrib broad, bright red (green below) surface with many small liver-coloured spots (fig. 12).

Stem erect or very slightly bent at tip, 120–125 cm., numerous basal branches widely decumbent then arcuate-ascending, about as long as central stem, cauline branches few and short. Stem conspicuously ribbed, bright deep red, very finely appressed-puberulous, with very sparse long hairs from papillae which are red where exposed to light. Lower stem leaves deflexed, upper spreading, shape

narrowly oblong-lanceolate or elliptic-lanceolate, 14–17 cm. \times 28–33 mm., \pm concave, margin finely reddish-denticulate, scarcely wavy, surface sparingly purple-blotched, usually smooth, midrib deep red; upper surface extremely finely, lower surface very finely, appressed-puberulous. Lower bracts long and leafy, spreading or spreading deflexed, reaching 10 cm. \times 24 mm. Upper bracts much shorter, spreading or patulous, *ca.* 40–45 \times 10 mm. Apex of inflorescence flat or slightly concave, wide, conspicuously comose, overtopping developed buds.

Ovary 13–15 \times 3–3.2 mm., ascending erect hispid from green papillae and densely spreading glandular-pubescent. Hypanthium 27–40 \times *ca.* 2.5 mm., sparsely ascending hispid and rather sparsely glandular-pubescent. Bud-cone yellowish-green,

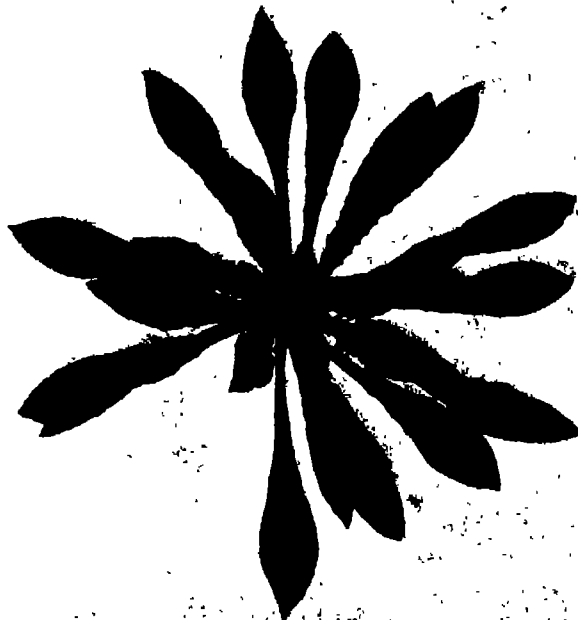


FIG. 12—*O. comosa*, rosette, culture 23.34.

quadrangular, 12 \times 5.5–5 mm., rather abruptly attenuate, very sparsely ascending hispid, shortly finely subappressed-pubescent, and rather densely spreading glandular-pubescent. Sepal tips definitely subterminal, rigid, stout, erect, and separate, 4–5 mm. long, subulate, green (fig. 13). Petals 16 \times 15–16 mm., expanding to *ca.* 60°, overlapping, widely not deeply emarginate. Filaments *ca.* 10 mm., anthers 7 mm., overtopping stigma by 2–3 mm. or overtopped by stigma. Stigma lobes 4–6 mm., nearly parallel or opening out flat, base of stigma *ca.* 10 mm. above hypanthium. Fruits green, tapering from base to apex, \pm arcuate, 28 \times 5 mm., reaching 50 mm. long \times 8 mm. diameter near base, 3 mm. at apex which is strongly cupped, nearly glabrous below.

Diagnosis—Folia radicalia atroviridia, anguste elliptica, acuta ; costa lata, clara rubra ; superficies multibus parvis maculis purpuris. Caulis erectus aut suberectus clarus ruber, inflorescentiae apex comosus. Ovarium viridotuberculatum, petala 16 mm. longa, apices sepalorum robusti rigidi, subterminalis, erecti disjuncti.

It was at first intended to include this species as a variety of *O. novae-scotiae*, but the differences were too marked. This species shows evident relationships not



FIG. 13—*O. comosa*, in flower, culture 23.34.

only with *O. novae-scotiae* but also with *O. intermedia* and *O. Hazelae* var. *parviflora*. *O. comosa* is similar to *O. novae-scotiae* in general habit and in stem colouring, but it differs in (1) narrower dark green rosette leaves bearing many small liver-coloured spots, a few occurring also on the stem leaves. These leaf spots are found in a number of *Oenotheras* and may be due to a single gene ; (2) rosette leaves much smaller, \pm shining, midribs dark red ; (3) stems tall, sometimes very slightly bent at the tip ; very few short cauline branches ; (4) apex of inflorescence comose ;

(5) ovary bearing green papillae ; (6) sepal tips stout, rigid, subterminal, erect, and separate.

O. comosa resembles *O. intermedia* and *O. Hazelae* var. *parviflora* in having rosette leaves rather dark green and shining (but with more numerous liver-coloured spots) and in having the stem tip somewhat bent. They also all (as well as *O. novae-scotiae*) agree in having red or pink midribs. *O. Hazelae* var. *parviflora* differs from *O. comosa* in having much smaller flowers (petals 8×10 mm.), in habit, in the stouter, subterminal, separate sepal tips, different terminal bracts, etc. *O. comosa* resembles *O. intermedia* in the subterminal sepal tips, but differs from it in (1) narrower rosette and stem leaves, (2) very slightly bent stem tip, (3) almost complete absence of long hairs from papillae, (4) larger flowers and longer style. From culture 5.33 the catenation was determined by Mr. C. E. FORD as a ring of 14.

O. intermedia, n. sp.

This species is derived from seeds collected at Bear River, Digby Co., Nova Scotia, on 22 September, 1932. The following cultures were grown and were quite uniform:

1933	3 (50 plants)
1934	<u>21</u> (28)
1935	<u>50</u> (31)

Description—Rosette leaves green, rather bright and shining, oblanceolate to elliptic-oblanceolate, apex acute, or obtuse with an acute point, reaching 15–35 cm. \times 35–60 mm. (petiole 5–9 cm.), \pm crinkled and undulate, margin subentire, repand-dentate or even pinnatifid below, repand-denticulate above, with reddish teeth ; midrib wide, conspicuously, often rather deep, pink ; both surfaces finely appressed-pubescent, upper leaves usually with sparse liver-coloured spots (fig. 14).

Stem erect or stem tip (9 cm.) bent, *ca.* 60 cm., ribbed, green (generally no red papillae), with diffuse red in lower part, hirsute from mostly green papillae and patulous-pubescent, a ring of basal branches coming into flower before the main stem and becoming bright red above. Lowest stem leaves \pm pinnatifid below, middle leaves 21 cm. \times 42 mm., petiolate, red below at stem attachment, midribs pink, \pm horizontal, \pm troughed, indumentum as rosette, but midrib has also scattered, patulous pubescence below. Lower bracts lanceolate, acute, 10 cm. \times 29 mm. Upper bracts 25 \times 9 mm., erect, slightly tipped with red. Inflorescence dense, flat-topped, bracts exceeding the developing buds (fig. 15).

Ovary 10–14 \times 3 mm., numerous long ascending hairs with scarcely developed papillae, short erect and \pm appressed pubescence. Hypanthium 22–29 \times 2 mm., with sparse long hairs and scattered short pubescence. Bud-cone 9–11 \times 5 mm., greenish-yellow, squarish, not tapering, indumentum as ovary but long hairs more spreading, from green papillae. Sepal tips 2–5 mm., markedly subterminal, arcuate,

erect or \pm spreading, green tipped with red. Petals $10-14 \times 10-18$ mm., emarginate, opening to 45° , overlapping. Filaments 7-9 mm., straight, anthers 6-7 mm., reaching nearly to top of stigma. Stigma lobes 4-5 mm., separating in anthesis, base of stigma 3 mm. above hypanthium. Fruits 25×6 mm., green, tapering from base to apex, sparsely hirsute without papillae, and with scattered pubescence.

Diagnosis—Folia radicalia viridia, aliquantum clara et nitentia, oblanceolata aut elliptico-lanceolata, acuta aut obtusa cum apice acute, \pm bullata et undulata, margine subintegro, repando-dentato aut pinnatifido ad basim, repando-denticulato



FIG. 14-- *O. intermedia*, rosette, culture 3.33.

supra, dentibus rubescentibus; costa lata, manifesto rubicunda. Caulis erectus aut apex declinatus, viridis cum diffuso rubro colore ad basim. Folia caulina infima \pm pinnatifida ad basim, folia petiolata, rubra infra ad nodum, \pm horizontalia, concava. Inflorescentia spissa, bractae alabastrae excedentes. Ovarium multis pilis longis ascendentibus ex tuberculis vix formatis, apices sepalorum admodum subterminales, arcuati, erecti vel \pm expansi, petala 10 mm. longa, basis stigmatis 3 mm. supra hypanthium.

This species shows such marked differences from *O. novae-scotiae* and *O. Hazelae*, to both of which it is clearly related, that it has been necessary to describe it as a separate species. It agrees with *O. novae-scotiae* in having the lowest stem leaves pinnatifid below, and it resembles that species in many of its characters. The differences, however, are marked and extend to all parts of the plants. They include (1) the

narrower, shiny rosette leaves, (2) the weakly bent stem tips, *O. novae-scotiae* always having an erect stem, (3) the smaller flowers, (4) the much later development, (5) the subterminal sepal tips, (6) the very short style. It resembles *O. Hazelae* var. *parviflora*, which also occurs in adjacent areas, perhaps more nearly, especially in flower-size, in habit (short stems with long basal branches coming into flower first), in its shining rosette leaves with purplish spots; but it agrees with the much more distinct *O. Hazelae* from eastern Nova Scotia rather than the var. *parviflora*



FIG. 15—*O. intermedia*, in flower, culture 50.35.

in (1) the bent stem tip, (2) the short style just above the mouth of the hypanthium, (3) the longer, erect, subterminal sepal tips.

This species thus combines features of both the species mentioned, while disagreeing with both. It has perhaps originated through crossing, though not as a simple hybrid between these species, but it breeds true owing to the catenation of its chromosomes, and it cannot logically be treated as a mere variety of either *O. novae-scotiae* or *O. Hazelae*. On the other hand, the three forms cannot be merged into one variable species because *O. novae-scotiae* and *O. Hazelae* maintain their separate identity when growing in the same areas, as at Middleton, N.S. The name *O. intermedia* is used in default of a more appropriate one.

O. flecticaulis n. sp.

From seeds collected on a point of land and shingle beach near the mouth of the Lahave River, Lunenburg Co., N.S., on October, 1932, by Mrs. WINTHROP BELL. The following cultures of this striking species were grown :

1933	78 (50 plants)	79 (50)
1934	103 (10)	
1935	102 (5)	



FIG. 16—*O. flecticaulis*, rosette, culture 78.33.

Description—Rosette leaves rather shining, greyish-green, narrowly elliptic-oblongate, apex acute or shortly cuspidate, reaching 15-19 cm. \times 23-32 mm. (in 1935, 19-26 cm. \times 45-50 mm.), including petiole 5-7 cm. long, flattish or slightly concave, crinkling slight, or definite near midrib, wavy or not, midrib white to pink, margin subentire to repand-dentate below, repand-denticulate above, teeth reddish, both surfaces very finely closely appressed-puberulous (fig. 16).

Stem short, very strongly bent (fig. 17), very red, with many red papillae, strongly ribbed, basal branches as long as main stem, many cauline branches. Stem leaves narrow (midleaf 10 cm. \times 19 mm.), crinkled, troughed, pendant, margin wavy, repand-denticulate below, subentire above. Bracts narrow but long, troughed, \pm curled. Inflorescence compact, apex flat, comose. Ovary 9 mm., hirsute

from small papillae which are red on exposed side of ovary, and glandular-pubescent. Hypanthium 29×2 mm., may be streaked with red where exposed, scanty long hairs. Bud-cone 12×6 mm., squarish, yellowish, tapering from base to apex, with red papillae in streaks, especially along the midveins, covered with exceptionally long hairs from papillae, some of which are red. Sepal tips terminal, green, appressed, 4-5 mm. Petals 9-12 \times 8-11 mm., overlapping, not opening out flat, with broad shallow sinus. Filaments 9-10 mm., anthers 4 mm. Stigma lobes



FIG. 17—*O. flecticaulis*, in flower, culture 103.34.

6-7 mm., appressed, style about 1 mm. above hypanthium. Fruits 30×8 mm., green, many short and scattered long hairs especially towards apex.

Diagnosis—Folia radicalia aliquantum nitentia, canoviridia, anguste elliptico-oblongata, acuta aut breviter cuspidata, leviter bullata, undulata aut non, costa alba ad rubicunda, margine subintegro ad repando-dentata ad basim. Caulis brevis, apex admodum flexus, valde ruber, rubropapillatus. Folia caulina angusta, bullata, pendentia. Inflorescentia compacta, apex planus, comosus. Ovarium 9 mm. longum, hirsutum cum papillis parvis rubescentibus ubi lucem

accipit, glandulariter pubescens. Hypanthium rubrolineatum ubi lucem accipit. Alabastra flavescentia cum rubris papillis lineatis, apices sepalorum terminales, virides, appressi, 4-5 mm. longi. Petala 9-12 mm. longa, 8-11 mm. lata.

This species belongs to the series of forms with strongly bent stem tip and numerous red papillae sensitive to light, which appear to extend around the eastern coast of Canada. They include *O. ammophiloides* from Guysborough Co., N.S., its var. *laurensis* from Westmoreland Co., N.B., and *O. Hazelae* var. *parviflora* from



FIG. 18—*O. flecticaulis* mut. *linearis*, in flower, culture 79.33.

Shelburne and Lunenburg Counties, N.S. *O. flecticaulis* agrees in flower-size with its nearest coastal neighbour, the last of these forms, while *O. ammophiloides* and its variety have petals of about twice the length. The present species differs from *O. Hazelae* and its variety in a number of significant points, including (1) the strongly bent stems, (2) pale pink to white midribs, (3) red papillae on stem, (4) stem leaves narrower, crinkled, and troughed, (5) bud-cone tapering throughout and continued in the terminal, appressed sepal tips.

Culture 79.33 contained one mut. *linearis* (fig. 18) which was significantly similar to that from *O. ammophiloides* var. *laurensis* (fig. 39, p. 295), and was also doubtless

trisomic. The rosette leaves were 27 cm. \times 15 mm., the stem leaves linear, one leaf 22 cm. \times 4 mm., and the flowers much smaller with widely divaricating sepal-tips.

O. Hazelae n. sp.

From seeds collected in October, 1932, by Mrs. WINTHROP BELL, (a) by the railway on the mainland near Lockeport, Shelburne Co., N.S., and (b) by the railway track on an islet between Lockeport and the mainland. Seeds of the same species were collected by me at Wentworth, Cumberland Co., N.S., by the railway tracks near the station, on 28 September, 1932. The Wentworth strain agrees closely with that from Lockeport, but differs in certain minor particulars. This species is named after Mrs. WINTHROP BELL, of Chester, N.S., whose activity in collecting and obtaining seeds from various localities has been a great help in these investigations. It, with its var. *parviflora*, appears to be the most widely distributed species in Nova Scotia, being known already in five widely separated counties. It is a handsome species of small stature but with many distinctive features, and is described from culture 107.34 as the type. The following cultures (Table VII) have been grown and compared :—

TABLE VII

	Lockeport		Wentworth
1933	80 (44)	82 (50)	11 (50 plants)
1934	105 (45)	107 (15)	30 (17)
1935		104 (5)	56 (8) 57 (30)

Description—Rosette leaves pale greyish-green, ca. 14–15, oblanceolate or narrowly elliptic-oblanceolate, apex acute, obtuse, or apiculate, 16–24 cm. \times 26–56 mm. (petiole 3.5–5 cm.), flattish, no crinkling or undulation, except in younger leaves, sparse liver-coloured blotches, margin subentire, repand-dentate or pinnatifid below, repand-denticulate above, teeth red; midrib pale pink above and below; surface finely appressed-pubescent on both surfaces (fig. 19).

Stem erect or slightly bent, short, ca. 66–74 cm., numerous basal branches developing early and coming into flower before the central stem, decumbent at base then widely arcuate-ascending, equalling or exceeding the central stem, cauline branches none or few. Stem stout, very strongly broadly ribbed from leaf bases, red at base, paler above, green towards apex, branches dark red except upper third; stem spreading- or patulous-ascending-hirsute in two series of different length, from pale red or almost colourless papillae. Stem leaves spreading, lanceolate, or elliptic-lanceolate (lower \pm oblanceolate), 11–15 \times 30–38 mm., acute with reddish tip, conspicuously concave, not wavy, rather obscurely crinkled, margin strongly repand-dentate below in lower leaves, upper very sparsely and shallowly so, \pm

repand-denticulate throughout, teeth green or reddish, midrib \pm pink above, leaves sparsely and very finely appressed-puberulous on both surfaces, midrib with a few longer appressed or patulous hairs. Lower bracts spreading, very concave, lanceolate, 8–10 cm. \times 28–33 mm. Upper bracts large, spreading, or patulous, very concave, 2.5–4 cm. \times 6–13 mm., with bright red tips, forming a characteristic terminal rosette. Inflorescence compact, up to 25 cm. long apex flat, very slightly depressed, comose, exceeding the highest developed bud-cones, broad and dense (fig. 20).

Ovary 11–12 \times 3.5–5 mm., densely ascending-hirsute with white or small red papillae and green glandular pubescent. Hypanthium 28–34 \times ca. 1.8 mm.,

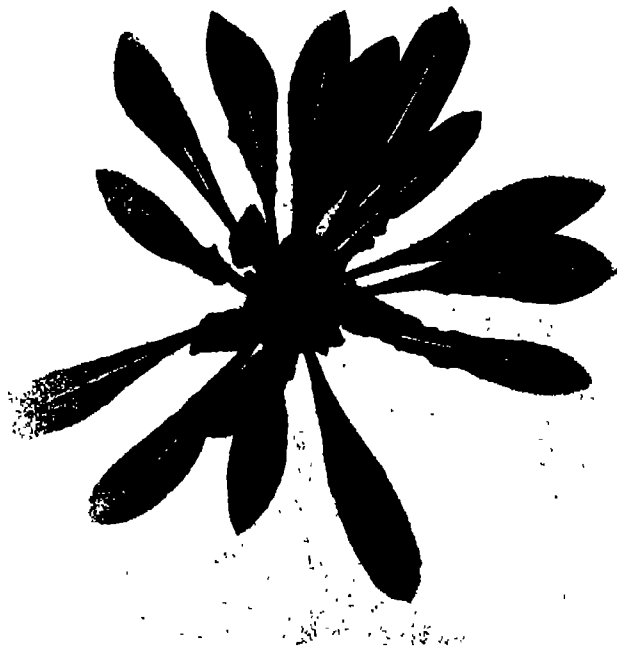


FIG. 19—*O. Hazelae*, rosette, culture 30.34.

sparsely patulous-hirsute and spreading glandular-pubescent. Bud-cone yellowish, squarish 12–14 \times 5–5.5 mm., rather densely patulous-hirsute with white papillae, and densely spreading-glandular-pubescent. Narrowed abruptly to sepal tips 4–4.5 mm., green throughout or slightly tipped with red, hooded inside, erect or somewhat divergent. Petals ca. 14–16 \times 18–20 mm., scarcely or slightly overlapping, thin and delicate, opening nearly flat, deeply widely emarginate with sinus ca. 2 mm. deep. Filaments 10 mm., slightly arcuate, anthers 6–8 mm., reaching top of stigma in bud, base of stigma 1–8 mm. above hypanthium tube, stigma lobes 3–6 mm., appressed or spreading. Flowers very fragrant. Fruits 26 \times 7 mm., tapering above, square, green, with few short and sparse longer hairs.

Diagnosis—Folia radicalia pallida cano-viridia, oblanceolata aut anguste elliptico-oblanceolata, acuta, obtusa aut apiculata, plana, sparse purpureo-maculata; costa pallida rubicunda supra et infra. Caulis brevis, erectus vel leviter declinatus, multis ramis radicalibus longis instructus qui ante caulem proprium florescunt. Bractae superiores grandes, patulae, valde concavae, singularem rosulam terminalem efficientes. Inflorescentia compacta, apex admodum leviter depressus comosus



FIG. 20—*O. Hazelae*, in flower, culture 105.34.

latus, densus. Ovarium dense hirsutum, albopapillatum, alabastra flavescentia, albopapillata, subito angusta ad apices sepalorum quae sunt virides, intus scapulati, erecti aut aliquantum divergentes. Petala 14–16 mm. longa.

This distinct species bears some resemblance to *O. novae-scotiae* in its marked red colouring but is by no means nearly related to it, and no other species is at all like it. The outstanding specific characters are (1) the habit, with very neat rosettes, basal branches often exceeding the short stem and coming into flower first, (2) the terminal

rosettes of stem and branches with relatively large, smooth bracts, (3) the fragrance was very marked in cultures 80.33, 82.33 and 107.34, but the third generation (104.35) showed no fragrance.

The cultures of this species were very uniform but culture 104.35 contained one probable mutant, smaller with shorter leaves, and paler midribs. In culture 80.33 one flower was tripartite, with 3 petals, 3 sepals, 6 anthers symmetrically



FIG. 21—*O. Hazelae*, in flower, culture 30.34.

arranged, and stigma lobes which might be counted as 3 or 4. The Wentworth strain differed in (1) tips of stem and branches strongly bent, (2) flower smaller (petals 10-11 \times 10-14 mm.), (3) sepal tips shorter (2 mm.), markedly subterminal (fig. 21).

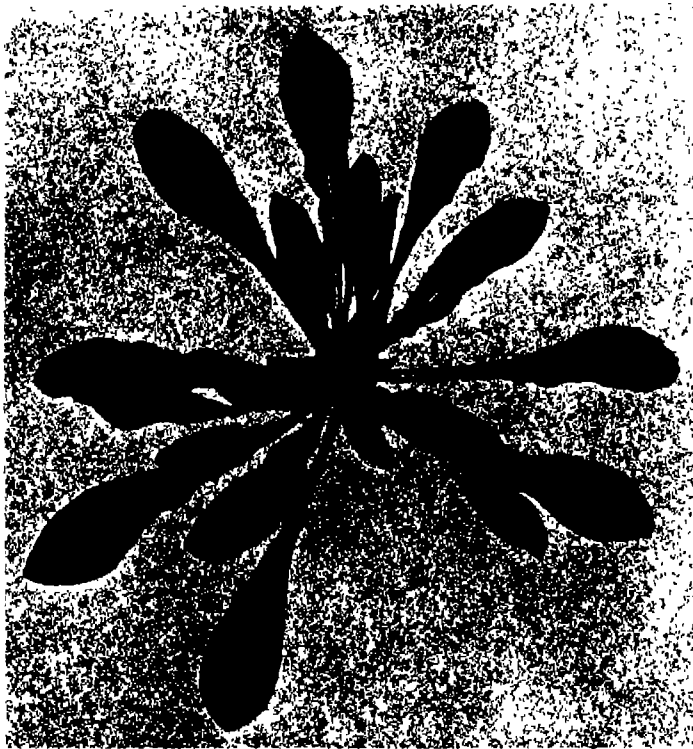
O. Hazelae var. *parviflora* n. var.

From seeds collected by Mrs. WINTHROP BELL at Port Mouton, Queen's Co., N.S., and Chester, Lunenburg Co., N.S., in October 1932. The first two plants showed

many basal branches and were growing on the sand dunes at Port Mouton, the third also growing on the coast. The Middleton strain was collected by me on 26 September, 1932. It was noted that some rosettes in the small colony had bright red midribs and some green. The sepal tips were also noticed to be "sprung" and red inside at the base.

TABLE VIII

	Port Mouton	Port Mouton	Chester	Middleton
1933	87 (50 plants)	88 (50)	89 (50)	7 (50)
1934	110 (59)	111 (47)	112 (25)	26 (6)
1935	105 (32)	106 (5)	107 (32)	54 (18)

FIG. 22—*O. Hazelas* var. *parviflora*, rosette, culture 107.35.

The two sets of cultures from Port Mouton are practically identical, while those from Chester differ in certain particulars to be mentioned. They all agree with the species in habit (short stem and a ring of longer basal branches coming into flower first), rosette leaves elliptic-oblanceolate, midribs pink, sparse liver-coloured blotches, and in general flower characters. They differ, however, in having longer, narrower rosette leaves (19-26 cm. \times 30-42 mm.) \pm crinkled, dark green, shining, midribs brighter pink, wider leaves (19 cm. \times 49 mm. against 14 cm. \times 34 mm.), stems and midribs much redder. The stem tip is sometimes distinctly bent but sometimes erect. The petals are smaller, 8 \times 10 mm., opening out flat, almost

truncate, stigma lobes 1–2 mm., sepal tips shorter (1–2 mm.). These measurements are from cultures 110.34 and 105.35. In comparing cultures 88.33 and 89.33, the Chester plants were found to differ in the following particulars : (1) Leaves somewhat larger and rosette leaves lighter green, less crinkled, more spatulate (fig. 22), (2) stem green, basal branches weakly red, no red papillae, (3) flowers somewhat larger, petals 10×12 mm. These locality differences have been maintained in two later generations of cultures. This is an example of the way in which small but constant differences are frequently found when the same species or variety is grown from adjacent geographic areas.

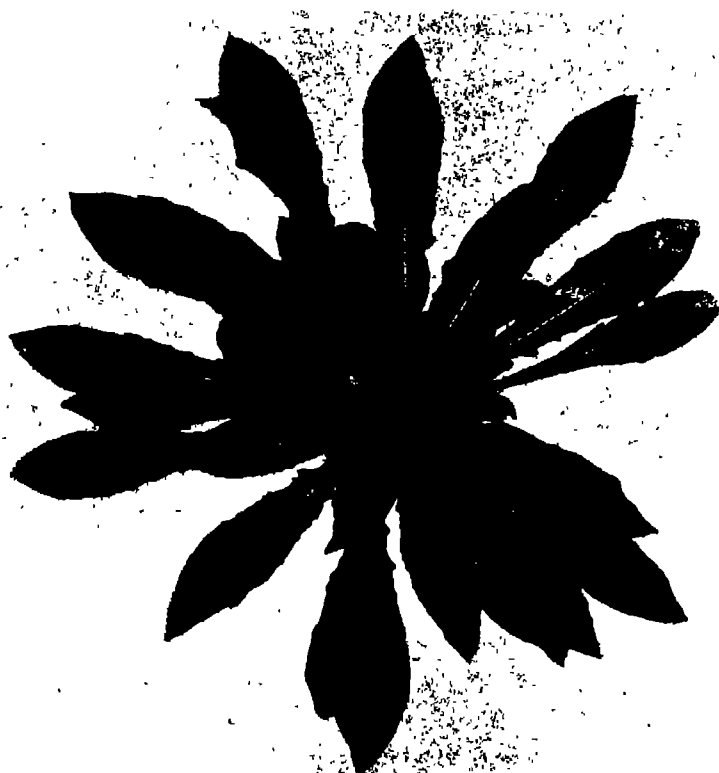


FIG. 23—*O. Hazelae* var. *parviflora*, rosette, culture 26.34.

Diagnosis—A species sic differt : folia radicalia longiora, angustiora, \pm bullata, atroviridia, nitentia, costae latiores, clariores rubicundae ; caulis rubrior ; petala minora (8 mm. longa, 10 mm. lata) ; apices sepalorum breviores.

The Middleton strain agreed with the variety in having dark green, shiny leaves with bright red midribs (fig. 23), but was in some respects intermediate between species and variety. The flower-size varied, petals $8\text{--}13 \times 10\text{--}15$ mm. The habit, with basal branches longer than the central stem and coming into flower first (fig. 24), is the same as that of the species. This strain was observed in 1935 to differ from the Wentworth cultures 56.35 and 57.35 in the following characters :

(1) stem erect, (2) foliage somewhat darker green, more crinkled, and with more liver-coloured spots, (3) last rosette leaves not spatulate, (4) stigma higher above hypanthium (at mouth of tube in the Wentworth strain), (5) sepal tips longer, subterminal but tips in contact (shorter and erect in Wentworth strain), (6) seedling has leaves less wide. Fig. 25 shows the central stem in flower (*cf.* fig. 20). From culture 87.33 Mr. C. E. FORD determined the catenation as a ring of 14.

It appears probable that if all the strains from intermediate areas could be assembled they might fill the gaps so as to give an appearance of essential continuity between the species and variety. Nevertheless, it is convenient at present to recognize the variety as distinct from the species. The seedlings, at least of the Middleton strain, have narrower leaves than in the species.



FIG. 24—*O. Hazelae* var. *parviflora*, habit, culture 54.35.

O. subterminalis n. sp.

From seeds collected from one plant in a colony by the roadside at Higgins Brook, near Wentworth and at North River, Colchester Co., Nova Scotia, 28 September, 1932. The plants at Higgins Brook were observed to have rather narrow, crinkled leaves, red midribs and stems, buds green. The cultures grown are shown in Table IX.

TABLE IX		
	Higgins Brook	North River
1933	13 (50 plants)	14 (50)
1934	33 (4) 34 (15)	35 (8)
1935	61 (3)	62 (11)

The original culture of 50 plants was uniform except for one rosette which was much larger and with broader leaves. This rosette was chimaeral, having some leaves which were pale green on one side of the midrib and normal green on the other. Open pollinated seeds from this plant yielded 4 rosettes in culture 33.34, which were uniform and narrower-leaved than the type, but may have been hybrids. The description is from culture 34.34.



FIG. 25—*O. Hazelae* var. *parviflora*, in flower, culture 26.34.

Description—Rosette leaves dull, rather deep greyish-green, oblanceolate, apex acute and shortly acutely cuspidate, reaching 11–14 cm. \times 27–40 mm. (petiole 3–4.5 cm. long), usually strongly concave, crinkling very conspicuous becoming bullate, midrib conspicuously pinkish, margin strongly repand-dentate to pinnatifid below, repand-denticulate above, teeth, margin, and apex reddish-purple, leaves finely appressed-pubescent on both surfaces, very sparsely purple-blotched (fig. 26).

Stem *ca.* 56–67 cm., tip bent, ring of basal branches red, decumbent then widely ascending, nearly as long as the central stem, tips bent. Stem ribbed, dark red, patulous-hirsute with red papillae, and appressed crispulous-puberulous, collar green. Stem leaves arcuate, deflexed, narrowly elliptic-lanceolate, upper lanceolate, acute with red tip, concave, crinkled, midrib deep pink, margin strongly repand-

denticulate below (lower sub-pinnatifid below), repand-denticulate above, teeth reddish or green, appressed-puberulous above and subappressed-puberulous below. Upper bracts 1.7–2.5 cm. \times 5–10 mm., spreading arcuate, red tips often recurved (fig. 27).

Inflorescence very compact, apex flat when young, convex when mature. Ovary 12×3 mm., densely patulous-hirsute and short spreading-glandular-pubescent, small pale, reddish papillae. Hypanthium $25-30 \times 2$ mm., indumentum as on ovary, long hairs sparse from scarcely visible papillae. Bud-cone yellowish with reddish marginal streaks, squarish, slightly tapering, $14-15 \times 5$ mm., indumentum as ovary but short glandular hairs sparse and obscure. Sepal tips 5 mm., slender, erect, markedly subterminal (distinctly hooded inside) erect, neither appressed nor



FIG. 26—*O. subterminalis*, rosette, culture 13.33.

spreading, greenish or tipped with red on inner face. Petals 20×24 mm. (12×15 mm. in 1933),* truncate and very obscurely emarginate, overlapping at base, opening out flat and not wilting quickly. Filaments 12 mm., anthers 8 mm., overtopping stigma by at least 4 mm. Stigma lobes 2–4 mm., gradually spreading or appressed, base of stigmas 8 mm. above hypanthium. Fruits somewhat tapered, \pm red in upper part, ca. 22×5 mm., base nearly glabrous, many long and short hairs on upper part.

Diagnosis—Folia radicalia surda satis plena cano-viridia, oblanceolata, acuta et breviter acute cuspidata, insigniter bullata, costa manifesto rubicundescens. Caulis brevis, atroviridis, apice declinato, ramis radicalibus longis instructus. Folia caulina arcuata, deflexa, anguste elliptico-lanceolata, acuta, apicibus rubris, concava,

* All the flower measurements in this culture were larger in 1934 than in 1933. This may have been because the plant selected as parent of culture 34.34 happened to have genes for somewhat larger flowers.

bullata, costa atrorubicunda. Ovarium 12 mm. longum, 3 mm. latum, tubercula parva rubescentia ; alabastra flavescentia rubrolineata. Apices sepalorum manifesto subterminales, erecti. Petala circa 20 mm. longa.

This species is well characterized by its somewhat narrow, crinkled leaves, with red midribs, red stem, and flowers of moderate size with markedly subterminal sepal tips, the latter feature almost as marked as in *O. angustissima*, to which it is, however, not nearly related except in this character. It resembles *O. Hazelae* in the



FIG. 27—*O. subterminalis*, in flower, culture 34.34.

short \pm red stem slightly bent at tip, but differs in the narrower, crinkled, acute-pointed leaves and smaller bracts. It does not appear to be closely allied to any other species. From culture 13.33, Mr. C. E. FORD determined the catenation to be a ring of 14.

The North River strain, observed in three generations, showed certain minor constant differences : (1) somewhat smaller plants with narrower less crinkled leaves, (2) bright pink midribs, (3) smaller, conspicuously emarginate-notched petals (14×14 mm.), withering more quickly. The two strains agree in all other characters.

O. grandifolia n. sp.

From seeds collected at Wentworth Station and Port Howe, Cumberland Co., N.S., and Waugh's River, near Tatamagouche, Colchester Co., N.S., on 28 September and at Point de Bute, Westmoreland Co., N.B., on 30 September, 1932. This species forms very large rosettes and evidently occupies a considerable area in eastern Nova Scotia and the adjacent portion of New Brunswick. It has been studied in the cultures shown in Table X.



FIG. 28—*O. grandiflora*, rosette, culture 31.34.

TABLE X

	Wentworth		Waugh's River	Port Howe	Point de Bute
1933	12 (50 plants)		15 (50)	16 (50)	21 (50)
1934	31 (15)	32 (14)	36 (5)	37 (15)	44 (26)
1935	58 (22)	59 (13)		63 (21)	

Description—Rosette leaves light green, 11-21 in number, elliptic to obovate-elliptic, apex acute or shortly acutely cuspidate, very large, reaching 22.5 cm. × 65 mm. (petiole 2.5-4 cm. long), flattish, ± crinkled, conspicuously undulate, margin repand-dentate or almost runcinate below to repand-denticulate above.

teeth green, except near apex which is usually purplish, midrib white with occasional faint tinge of pink, lamina usually very sparsely purplish-blotched, indumentum on both surfaces subappressed-pubescent (fig. 28).

Stem erect, short, *ca.* 75 cm., strongly ribbed above, ascending hirsute from green papillae and patulous- or appressed-puberulous. Usually a ring of basal branches. Stem leaves broadly lanceolate, dentate-pinnatifid below, then repand-dentate,



FIG. 29—*O. grandiflora*, in flower, culture 15.33.

repand-denticulate, apical third subentire, lowermost 28 cm. \times 72 mm., margin wavy, teeth green, midribs white, both surfaces patulous-puberulous, denser on midrib and scattered hirsute on midrib below. Lower bracts 10 cm. \times 26 mm., lanceolate, concave, margin undulate, distantly repand-denticulate. Upper bracts *ca.* 22 \times 5 mm. (fig. 29).

Inflorescence dense, apex flat, somewhat comose. Ovary 10–16 \times 3.5–5 mm., patulous-hirsute from green papillae and patulous-pubescent. Hypanthium 23–32 \times 2.5 mm., stout, greenish, scattered patulous-pubescent and erect short glandular-pubescent. Bud-cone greenish, 22 \times 5 cm., squarish, scarcely tapering, bearing long patulous hairs from colourless papillae and short suberect glandular

pubescence. Sepal tips 2-4 mm., terminal, appressed, apices spreading. Petals 22×21 mm., opening to 45° , overlapping, truncate, fading red at base only. Stigma lobes 8 mm., widely spreading. Anthers 10 mm., filaments arcuate, 14 mm., base of stigma lobes 10-11 mm. above mouth of hypanthium. Fruits long and slender, 39×5 mm., green, with many long and few short hairs.

Diagnosis—Folia radicalia ingentia, leucoviridia, elliptica ad obovato-elliptica, acuta aut breviter acute cuspidata, ad 22.5 cm. longa, 65 mm. lata, manifesto undulata, margine paene runcinata ad basim, dentes virides, costa alba, lamina plerumque rarissime purpura maculata, pubescentia subappressa. Caulis brevis, viridi-tuberculatus, folia caulina late lanceolata, dentata-pinnatifida ad basim. Inflorescentia densa, apex planus aliquantum comosus. Ovarium 10-16 mm. longum, 3.5-5 mm. latum, viridi-tuberculatum, hypanthium robustum, alabastra sub-viridia, apices sepalorum 2-4 mm. longi, terminales, appressi, apicibus extensis. Petala 15-24 mm. longa, truncata, in marcescendo rubra ad basim.

This species is most sharply characterized by its huge rosettes and very broad leaves. It shows certain resemblances to *O. pycnocarpa* and *O. novae-scotiae*, but differs from both in its short stems as well as in leaf and flower characters. The subruncinate leaves of the rosettes show some similarity to those of *O. pycnocarpa*. It is further distinguished from *O. novae-scotiae* by the almost complete absence of red pigment from stems and buds. The species shows marked resemblance to *O. biennis* L., from which it differs mainly in (1) much larger rosettes with broader leaves, (2) short stems. The Point de Bute strain (culture 21.33) was found, however, by Mr. C. E. FORD to have a ring of 14 chromosomes whereas the European *O. biennis* has a ring of 6. *O. grandifolia* appears to be nearer to *O. biennis* than to any other species. They agree in having no red papillae on the stem.

The seeds for the Wentworth cultures were collected 465 feet above sea level near the Railway Station, where there were clusters of small plants with broad leaves. From the seeds for culture 12.33 50 plants were grown, 34 of which were typical *O. grandifolia* while the remaining 16 differed in having narrower, paler green leaves, with bright pink midribs, and were earlier in development. This is probably a case of segregation, because it is repeated in other cultures belonging to *O. grandifolia*. The second type apparently belongs to *O. novae-scotiae*, which is found in the Annapolis Valley. A plant of this type was selfed to produce culture 32.34, in which the plants which flowered early were very much like *O. novae-scotiae*, while the persistent rosettes forming short stems were very much like *O. grandifolia*. Open-pollinated seeds from one plant produced culture 59.35 which again showed "segregation", 11 plants having narrower leaves and pink midribs while 2 had broad leaves and white midribs. The genetical relationship of *O. novae-scotiae* to *grandifolia* requires further investigation. Phenotypically they are very unlike.

The Waugh's River strain of *O. grandifolia* differs from the Wentworth strain in having the leaves much crinkled, as in *O. Lamarckiana*, and flowers slightly smaller (petals 18×18 mm. as against 21×21 mm. at end of 1934 season, but still smaller,

14 × 12 mm., in the previous year) with red papillae on the ovary, the rosette leaves reaching 27 cm. × 80 mm. Culture 15.33, like 12.33, contained plants of two types, three plants with pink midribs being classed definitely with *O. novae-scotiae*, the remainder being a much crinkled *O. grandifolia*. The Port Howe strain was uniform with somewhat crinkled leaves and pink midribs. The plants were strikingly like the type of *O. biennis* L., differing mainly in the crinkling, and the short stems. In this strain also the flower-size increased from petals 15 × 14 mm. in 1933 to 24 × 24 mm. in 1934 and 21 × 29 mm. in 1935. This species appears to show quite exceptional fluctuations in flower-size, one plant of culture 16.33 having petals 20 × 25 mm. The Port Howe and Point de Bute strains agree with *O. grandifolia* in having no red papillae on stem or ovaries, the latter agreeing with the type in every particular.

Striking features of this species are (1) its similarity to *O. biennis* L., (2) its "segregation" of a type resembling *O. novae-scotiae*, (3) its similarity in certain respects to *O. pycnocarpa*, although there is not a close relationship and the species is very distinct.

O. Royfraseri n. sp.

From seeds collected at Sackville, New Brunswick, by Professor ROY FRASER, in 1933. The cultures, grown at Regent's Park, are shown in Table XI.

TABLE XI

1934	1 (12 plants)	2 (11)	3 (43)
1935	38 (6)		39 (22)

Description—Rosette leaves dull greyish-green, elliptic-lanceolate or oblanceolate, apex obtuse to acute or shortly acuminate, reaching 11–14 cm. × 30–40 mm., somewhat crinkled in lower part, margin repand-dentate below, repand-denticulate above, last rosette leaves sinuately pinnatifid below, midribs reddish; upper surface pubescent with suberect hairs, on lower surface midrib and lateral nerves coarsely, mesophyll finely pubescent (fig. 30).

Stem erect, 56–65 cm. high, no basal branches, few ascending cauline branches. Stem obscurely angular and ribbed from leaf bases, pale green, hirsute from red papillae and finely crispulous pubescent. Stem leaves arcuate-spreading or spreading-ascending, elliptic-lanceolate, acute, margin often incurved, strongly repand-dentate, or subpinnatifid below, repand-denticulate above, teeth green, measuring 9–14.5 cm. × 23–40 mm., midrib pinkish, upper surface suberect-pubescent, lower surface densely ± erect pubescent, the prominent midrib and nerves green, with hairs of unequal length, one series longer than that of the mesophyll. Lower bracts spreading, lanceolate, upper bracts arcuate or ascending, tips recurved.

Apex of inflorescence nearly flat, but lower buds slightly longer than central. Ovary 12–13 × 3 mm., sparsely hirsute with very small red papillae and arcuate-subappressed-pubescent. Hypanthium 25–30 × 1.5–2 mm., with long scattered

ascending-patulous hairs and short, spreading gland-tipped hairs. Bud-cone 14×5 mm., squarish, yellowish-green, with sparse, spreading long hairs, and short, spreading gland-tipped hairs. Sepal tips 3-4 mm., green, slender, appressed, apices spreading, terminal, subulate, hooded inside. Petals 13-18 \times 13-17 mm., cuneate, \pm widely emarginate, with narrow spaces between, opening at $45-60^\circ$. Filaments arcuate, 10.5 mm. long, anthers 7-8 mm. Stigma lobes 4.5-6 mm. long, spreading, base 3-4 mm. above hypanthium, exceeded by anthers (fig. 31). Fruits green, nearly smooth.

Diagnosis—Folia radicalia surda canoviridia, elliptico-lanceolata aut oblanceolata, apex obtusus vel acutus vel breviter acuminatus, extrema folia sinuato-pinnatifida

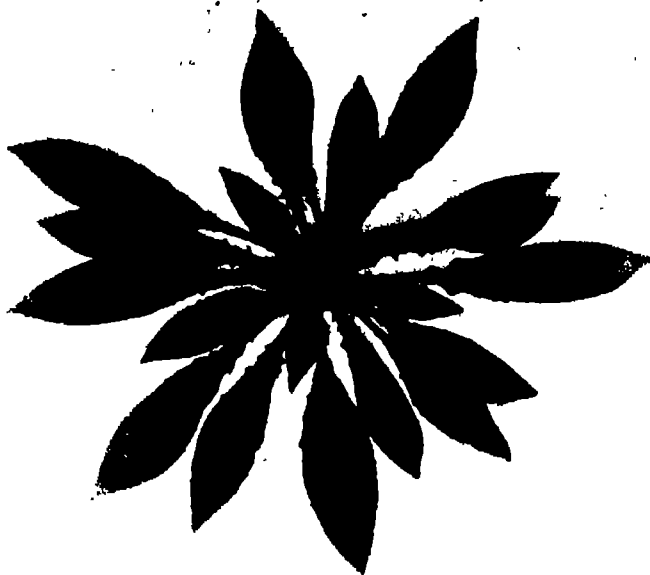


FIG. 30—*O. Royfraseri*, rosette, culture 1.34.

ad basim, costa rubescens. Caulis erectus, pallidoviridis, rubropapillatus, folia caulina arcuato-extensa, elliptico-lanceolata, repando-dentata ad subpinnatifida ad basim. Petala 11-18 mm., apices sepalorum viridia, attenuata, terminalia, apicibus extensis.

This species is markedly distinct from the other species, *O. sackvillensis*, found in the same locality. It differs from the latter in the following points: (1) reddish midribs, (2) *O. sackvillensis* has much smaller, sharper, more pointed and numerous basal teeth on all lower stem leaves, while in *O. Royfraseri* the last rosette leaves are sinuately pinnatifid below, (3) red papillae on stem, (4) sepal tips appressed, only the tips spreading, (5) petals smaller, 11-18 mm., (6) plants smaller, but this may be due to the 1934 cultures being somewhat shaded.

Culture 3.34 was grown in the partial shade of high trees, with the result that most of the plants were biennial, flowering in 1935, while the selfed seeds of one plant which flowered were grown in full sunlight as annuals (culture 39.35). The phenotypic effects of partial shade could thus be determined. The shade plants were somewhat smaller with somewhat narrower darker green leaves and paler red midribs. The petal-size does not appear to have been affected.

Culture 38.35 and 39.35 differed slightly, the former having many large red papillae on the stem and somewhat lighter green leaves, while the latter had few



FIG. 31—*O. Royfraseri*, in flower, culture 1.34.

small papillae. The former culture contained two plants which differed in having smaller petals (10–11 mm. instead of 15–17 mm.), redder stems and white midribs, while the latter culture contained one plant with equally small petals and no red papillae on the stem. These differences are presumably due to gene changes.

O. sackvillensis n. sp.

At Sackville, N.B., large colonies of *Oenothera* having uniform appearance were growing behind the power house of the University and in the adjacent vegetable

garden. Cultures from seeds collected 29 September, 1932, have been grown from this source, and are shown in Table XII.

TABLE XII

1933	17 (50 plants)		18 (28)	
1934	38 (3)	39 (4)		40 (12)
1936		64 (12)	65 (13)	66 (7)

FIG. 32—*O. sackvillensis*, rosette, culture 38.34.

Description—Rosette leaves light rather greyish-green, oblanceolate or elliptic-oblanceolate, apex acute or shortly cuspidate, blade reaching 18 cm. \times 55 mm. (one late rosette 28 cm. \times 64 mm.), petiole 4–6 cm. long, midrib white, slightly concave, considerably crinkled and undulate, margin repand-dentate or pinnatifid below, repand-denticulate above, teeth green, both surfaces of blade erect-pubescent, rare liver-coloured patches (fig. 32).

Stem erect (fig. 33), ca. 80 cm., basal branches long-decumbent or almost prostrate then widely arcuate-ascending, equalling or exceeding central stem; latter stout, ribbed below, green, no red papillae, sparsely shaggy, patulous-hirsute with hairs of varying length, densely \pm appressed crisped-pubescent. Middle stem leaves elliptic-lanceolate, smooth, somewhat wavy, strongly repand-dentate or subpinnatifid below, repand-denticulate above, teeth green, 17 cm. \times 44 mm., midrib white, hirsute below, blade suberect-pubescent both surfaces. Lower bracts spreading,

concave and wavy, narrowly lanceolate, *ca.* 4 cm. long, 9–10 mm. wide. Upper bracts ascending, flattish, *ca.* 2–2.5 cm. long.

Apex of inflorescence flattish, slightly depressed, far overtopping highest developed buds and flowers. Spike not dense. Ovary 13–17 \times 3.5 mm., rather densely patulous-hirsute from green papillae, and shortly spreading glandular-pubescent. Hypanthium 27 \times 3 mm., indumentum very sparse. Bud-cone greenish, squarish, tapering at apex, 16 \times 5.5 mm., indumentum as ovary. Sepal tips 3–4 mm.,

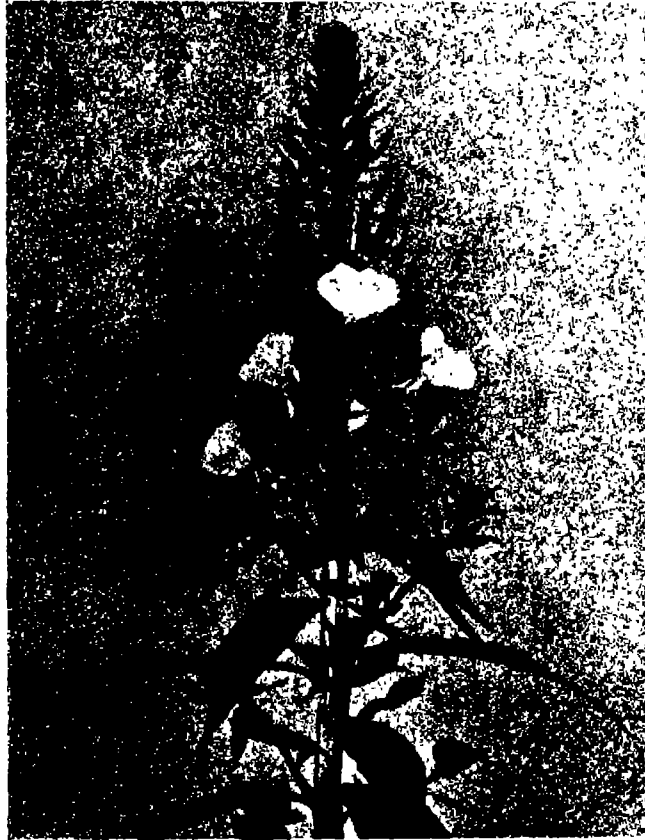


FIG. 33—*O. sackvillensis*, in flowers, culture 40.34.

green, terminal, appressed. Petals 21–25 \times 22–23 mm., opening widely, truncate and irregularly toothed, or obscurely retuse at apex. Filaments 13 mm., anthers 9 mm., about level with top of stigmas, lobes 6–7 mm. becoming widely divergent or remaining appressed, 7–9 mm. above hypanthium. Fruits green, *ca.* 30 \times 5 mm., bearing many long hairs, few short.

Diagnosis—Folia radicalia lucida subcanoviridia, oblanceolata aut elliptico-oblanceolata, acuta vel cuspidata, leviter concava, insigniter bullata et undulata, raris maculis purpureis, costa alba, margine repando-dentata aut pinnatifida ad

basim, dentibus viridibus. Caulis robustus, erectus, viridis, non rubropapillatus; inflorescentiae apex leviter depressus, flores longe superans. Ovarium robustum, 13-17 mm. longum, hirsutum, viridipapillatum; alabastra viridia, apices sepalorum 3-4 mm. longi, virides, terminales appressi. Petala 21-25 mm. longa.

This species is marked by its stout, erect stems, absence of red from papillae and midribs, and the medium sized flowers. In general leaf shape and flower-size and in the absence of red papillae from the stem it resembles *O. biennis*. It has shown striking variability, which may be briefly described. The culture 18.33 from wild



FIG. 34—*O. sackvillensis*, seg. *albiviridia*, rosette, culture 40.34.

seeds, which numbered 28 plants, included one dwarf mutation, which reached a height of *ca.* 60 cm. but remained unbranched, with small, curled bracts and smaller flowers (petals 17 mm.). This plant was selfed and produced culture 40.34, which included one dwarf exactly like the parent, 4 normal tall and 7 of a striking new type which may be called *albiviridia* n. var. (figs. 34 and 35) and which differs from the type as follows: the leaves are light green, rather conspicuously troughed, narrower (rosette leaves 10-17 cm. \times 25-33 mm.), conspicuously crinkled and undulate, with no liver-coloured spots, and the flowers appear to be somewhat deeper yellow.

Diagnosis—A specie sic differt: folia manifesto concava, albiviridia, angustiora, manifesto bullata et undulata, non rubromaculata.

Open-pollinated seeds of the dwarf in culture 40.34 yielded culture 65.35, thirteen plants, of which three were typical dwarfs (fig. 36), the rest being tall. This dwarf type proves on cytological examination by Mr. C. E. FORD to be a trisomic mutation, as its genetic behaviour suggests. A normal plant from culture 40.34 was selfed and gave seven plants, two of which were the light green type. Hence some normal



FIG. 35—*O. saskiellensis* var. *albiviridia*, in flower, culture 40.34.

plants segregate a high frequency of this type in each generation. Its chromosomes have not yet been examined. Culture 39.34 contained certain plants in which the green papillae on the sepals were elongated and cylindrical. One of these plants was selfed to produce culture 64.35, all of which were uniformly of the light green type. Hence it would appear that this segregate breeds true, and that the true character of the parent plant of this culture was overlooked.

O. ammophiloides var. *laurensis* n. var.

From seeds collected at Port Elgin, Westmoreland Co., N.B., near the railway station, on 30 September, 1932, and grown in Regent's Park. Cultures 19 and 20 were both from Port Elgin, while culture 22 was from the shore at Cape Tormentine



FIG. 36—*O. sackvillensis*, trisomic dwarf mutation, in culture 65.35.

and culture 23 was from seeds of a plant near C. Tormentine on the road to Port Elgin.

TABLE XIII

1933	19 (50 plants)	20 (22)	22 (50)	23 (50)
1934	41 (48)	42 (50)	45 (24)	46 (13)
1935	67 (35)	68 (6)		

Description—Rosette leaves thick, greyish-green, narrowly elliptic, later ones oblanceolate, apex acute or obtuse, reaching 34.5 cm. \times 52 mm. (petiole 4-7 cm.), margin repand-denticulate below, obscurely so above, with reddish glands, midrib white, both surfaces very finely appressed-pubescent (fig. 37).

Stem *ca.* 85-100 cm., tip bent (*ca.* 5 cm.), ring of basal branches shorter than central stem, tips bent, later becoming erect or suberect. Stem ribbed, ribs very thick above, thin below, stem pale green, or pinkish near base, patulous-ascending-hispid with bright red papillae, and finely arcuate-appressed puberulous.

Lower stem leaves deflexed, upper spreading, lower narrowly oblanceolate, upper \pm elliptic-lanceolate, 19-27 cm. \times 30-38 mm., acute, red-tipped, margin irregularly

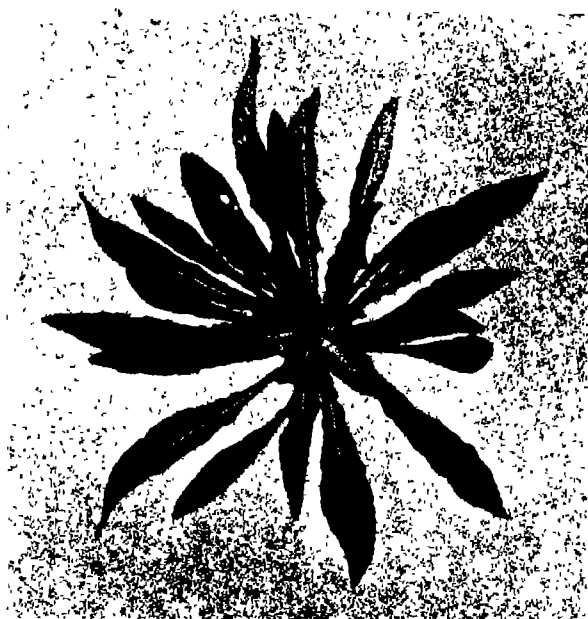


FIG. 37—*O. ammophiloides* var. *laurensis*, rosette, culture 22.33.

repand-dentate below, obscurely repand-denticulate above, upper half subentire, glands obscure, green, midrib white, with some longer \pm suberect hairs below, both leaf surfaces finely appressed-pubescent. Lower bracts lanceolate, concave, 12-13 \times 36-40 mm., spreading, uppermost ascending *ca.* 2.5 cm. \times 5 mm., red on under surface (fig. 38).

Apex of inflorescence flat or rather slightly depressed, comose. Ovary 10-13 \times 3 mm., rather shortly ascending-hirsute with many bright red papillae and shortly spreading glandular-pubescent. Hypanthium 28-33 \times *ca.* 2.5 mm., sparsely ascending- or patulous-hirsute from red papillae (papilla often forming a streak by extension of pigment longitudinally from its base) and sparsely spreading glandular-pubescent; hypanthium greenish, often tinged with deep red. Bud-cone squarish, scarcely tapering, yellowish, covered with conspicuous red papillae where exposed to light,

14-19 \times 5.5-6 mm., densely ascending-hirsute from red papillae and spreading glandular-pubescent, sepal tips 3-4 mm., subterminal, appressed or diverging, constricted at base, green with a red spot at the base inside. Petals 16 \times 16 mm.-22 \times 26 mm., emarginate, widely overlapping, opening to 45°, turning orange. Filaments 13 mm., anthers 7-8 mm., stigma lobes 8-11 mm., widely divergent, base of stigma lobes 4-6 mm. above hypanthium, anthers overtopping stigmas *ca.* 5 mm. Fruits 35 \times 7 mm., green with touches of red at apex, nearly glabrous



FIG. 38—*O. ammophiloides* var. *laurensis*, in flower, culture 19.33.

below, numerous mostly short hairs above. Mr. C. E. FORD examined a normal plant in culture 20.33 and found a ring of 14 chromosomes.

Diagnosis—A specie sic differt: flores grandiores (petala 16-22 mm. longa), stigmata longiora, fructus brevior, rubrae papillae, pauciores et minores, maxime in caule et hypanthio, caulis et alabastra minus pubescentia.

This form is clearly related to *O. ammophiloides* GATES and CATCHESIDE, which is also a coastal species, described from Guysborough Co., N.S. (GATES 1933, p. 180). It differs mainly from the latter species in (1) larger flowers, (2) longer stigma lobes,

(3) shorter fruits, (4) plant less hairy, (5) fewer and smaller red papillae, especially on system and hypanthium. It may therefore be regarded as a variety having larger flowers, fewer red papillae, less pubescence, and shorter fruits. Cultures 22 and 23 and their descendants differed from the type cultures 19 and 20 in the nearly complete absence of red papillae from stem and buds. This is a good example of the way in which minor differences appear in strains of the same species from different localities.

Culture 20.34, numbering 22 plants from wild seeds, produced one striking mutant *linearis* with extremely narrow, almost linear, subentire leaves (rosette leaves

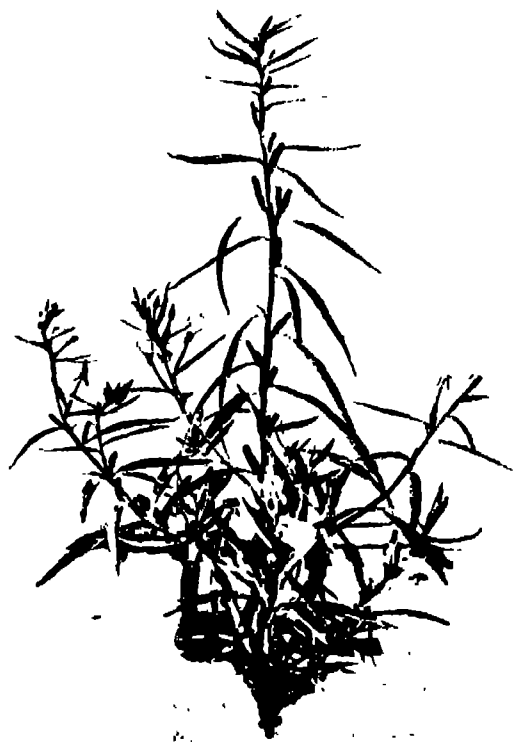


FIG. 39—*O. ammophiloides* var. *laurensis* mut. *linearis*, culture 43.34.

30 cm. \times 35 mm.) and smaller flowers (petals 13 \times 12 mm.). Otherwise it agreed with the type. Open-pollinated seed produced 3 plants (culture 43.34, two having very narrow leaves, one with white and the other with red midribs, showing that the latter was a hybrid, while the third, which flowered, had rosette and stem leaves only 5–8 mm. wide and empty anthers (fig. 39). This plant had even narrower leaves than the original parent mutant from which it was descended. The chromosomes were examined by Mr. C. E. FORD and it was found to be trisomic, having 15 chromosomes. Mut. *linearis* produces very few seeds, and from open-pollinated seeds of this plant only four offspring were obtained, two of which were

typical and two mut. *linearis*. Although not strongly viable, this linear-leaved trisomic has therefore reappeared in three successive generations with a frequency which is in accord with the 50 per cent expected, but it is not clear why the later specimens should have been so much more extreme than the original mutant.

This trisomic mutant is very similar to the mut. *graminifolia* obtained by RUDLOFF and STUBBE (1935) in the offspring of *O. Hookeri* after X-raying the pollen. It also had smaller flowers and mostly bad pollen. They class it with a number of others as a gene mutation. This conclusion is based on an examination of the pollen grains and the fact that when selfed they breed almost true, no count of the chromosomes being made. These reasons are, however, inadequate, and it appears more probable that mut. *graminifolia* was a trisomic. It is unfortunate that no cytological examination was made. MICHAELIS (1930) obtained 60 per cent heteroploid offspring from *O. Hookeri* by subjecting the plants to high temperature. These included plants with 15 and 13 chromosomes and others with a fragment, but unfortunately there is in this case no description of the phenotypic characters. By X-raying the pollen of *O. blandina*, CATCHESIDE (1935) obtained a number of F_1 variants. Four of these were called "willow leaf" and one "bootlace". The latter had rolled leaves about 1 cm. in diameter. Both types showed seven pairs of chromosomes and were probably due to deletions. On selfing or back-crossing to normal they gave all normal offspring. It thus appears that very narrow-leaved mutations may be (1) trisomic, (2) due to deletions, or (3) perhaps due to gene mutation.

O. parva n. sp.

This species is in a sense a continuation of the coastal species *O. ammophiloides*, which is found on the southern coast of Nova Scotia and continued on the gulf shore as var. *laurensis*. It occurs on the south shore of the St. Lawrence river and is represented by seeds collected on 2 October, 1932, from Bic, Rimouski Co., Quebec, by an old wharf; Trois Pistoles, Temiscouata Co., on the beach by the Biological Laboratory of Laval University; and at L'Islet, L'Islet Co., by the roadside near the river shore. The resulting cultures are closely similar, but they differ so markedly and constantly from the Port Elgin, N.B., cultures as to require separate specific recognition. The cultures grown are shown in Table XIV.

TABLE XIV

	Bic	Trois Pistoles	L'Islet	
1933	25 (50 plants)	26 (50)	27 (50)	
1934	47 (8)	48 (6)	49 (22)	50 (40)
1935		69 (26)	70 (30)	71 (30)

The relationship of this species to *O. ammophiloides* var. *laurensis* is confirmed by the seedling stage, which is very similar, the only detectable difference being the

constantly smaller size. It remains markedly smaller in the rosette and flowering stages. The L'Islet strain differed in certain features from the other two in the flowering stage, but as the rosettes were indistinguishable the description of this stage has been taken from culture 70.35 and that of the flowering plants from culture 47.34. The latter culture remained rosettes in 1934 and only flowered as (biennials) the following year. Minor differences between these strains will be pointed out later.

Description—Rosette leaves pale greyish-green, narrowly oblanceolate, acute, reaching 18 cm. \times 30 mm., smooth, concave, margin repand-dentate below, finely

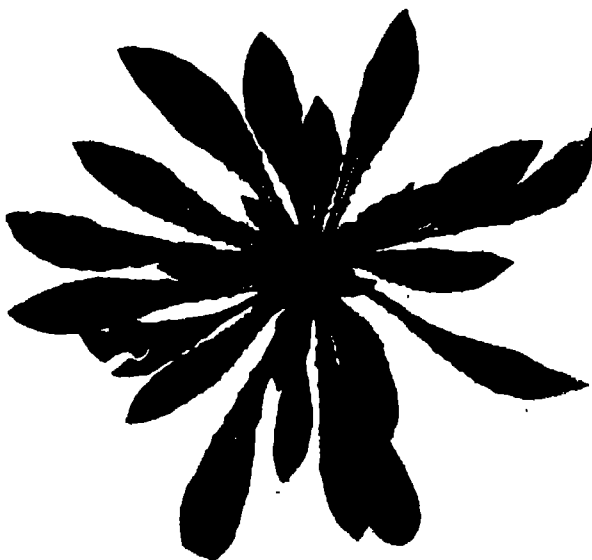


FIG. 40—*O. parva*, rosette, culture 47.34.

repand-denticulate above, teeth reddish, midrib white, indumentum on both surfaces of lamina extremely finely appressed-puberulous (fig. 40).

Stem reaching 140 cm. (in partial shade), 10 cm. at tip bent horizontal when developing, red from papillae, especially where exposed to light; basal branches decumbent then widely ascending, cauline branches numerous, arcuate-ascending. Stem thinly ribbed below, more conspicuously in upper half, usually red below, pale green and reddish above, softly conspicuously patulous-hirsute from crimson papillae and inconspicuously subappressed-cripsed-puberulous.

Stem leaves arcuate-deflexed, narrowly lanceolate or elliptical-lanceolate, flattish, margin dentate below with 1-3 coarse teeth, otherwise extremely finely denticulate with green or red teeth, 12.5-13.5 cm. \times 22-24 mm., midrib white, lamina very sparsely irregularly suberect-pilose and very finely densely \pm appressed-pubescent. Inflorescence with long internodes below, more densely flowered above, with few

flowers. Lower bracts patulous or deflexed, lanceolate, concave, wavy near base, *ca.* 5.5–8 cm. \times 14–20 mm. Upper bracts spreading with arcuate-upcurved apices *ca.* 2–2.5 cm. \times 5–6 mm. Apex of inflorescence flattish or slightly depressed, comose, *ca.* 1.5–2 cm. across, slightly overtopped by highest developed buds, straight (fig. 41).

Ovary 13 \times 4 mm., copiously ascending and patulous pilose from papillae which are crimson, especially on side exposed to light, densely shortly spreading, glandular-pubescent. Hypanthium 21–25 \times 2.5 mm., greenish, sparsely ascending pilose



FIG. 41—*O. parva*, in flower, culture 70.35.

from papilla bases which form \pm conspicuous red lines where exposed, copiously spreading glandular-pubescent. Bud-cone yellowish-green, red where exposed, quadrangular, *ca.* 10 \times 5 mm., densely ascending-pilose from numerous red papillae and shortly spreading gland-tipped-pubescent. Late buds and stem apex deep red, but uppermost bracts with little red on their lower surface. Sepal tips 4 mm., subterminal, green tipped with red, \pm divergent. Corolla cup-like, petals 16 \times 15 mm. (rapidly diminishing to 8–9 \times 6 mm.), emarginate, opening to *ca.* 60°, scarcely overlapping in early flowers, widely overlapping in later. Base of stigma

5-6 mm. above mouth of hypanthium, stigma lobes 7-2 mm. long, generally tipped with red, reaching 3 mm. above anthers, appressed or spreading. Anthers 7 mm., filaments 9 mm. ? Fruits 30×7 mm., red at tip when maturing.

Diagnosis—Folia radicalia pallida canoviridia, anguste oblanceolata, acuta, plana, costa alba, lamina magnopere tenuiter appresso-puberula. Apex caulis horizontaliter flectus, sanguineopapillatus. Folia caulina arcuato-deflexa, anguste lanceolata vel elliptico-lanceolata, plana. Inflorescentia paucis floribus; ovarium 13 mm. longum, 4 mm. latum, abundanter pilosum de papillis sanguineis, maxime ubi lacum accipit. Hypanthium viridescens, manifesto rubrolineatum, sepala flavescentia-viridia, rubra ubi lucem accipiunt. Petala 16-19 mm. longa, apices sepalorum 4 mm. longi, subterminales, virides, acuminibus rubris, \pm deflectentes.

The Bic culture 47.34 showed one interesting mutant. In the rosette stage the plants were uniform and differed from the Cape Tormentine strain (*O. ammophiloides* var. *laurensis*) only in having leaves narrower and smoother. Having wintered over, and formed stems about 6 inches high, in one plant the stem and leaf margins were seen (26 April, 1935) to be bright yellow, lacking chlorophyll except in the middle portion of the leaves. This periclinal chimera, unlike all other plants in the culture, was severely affected by a heavy spring frost. It managed to survive in a weak state, but all the yellow tissue disappeared, and it produced a few flowers as a green plant with narrow leaves and no trace of yellow tissue. Whether its seeds will germinate remains to be seen.

The strain from Trois Pistoles differs but slightly from the Bic strain in having the stem tips more strongly bent, the stems and buds somewhat redder. A plant from the Bic strain (culture 25.33) was examined by Mr. C. E. Ford and found to have a ring of 14 chromosomes. The L'Islet strain stands apart from the other two in several features: (1) rosette leaves less grey-green, margin of leaves redder, rosettes less persistent, (2) stem leaves shorter and narrower, less dentate below, stem suberect, short (reaching ca. 71 cm.), and slender, (3) plants earlier to finish flowering, producing only about 15 (as against 30) flowers on the main stem, (4) stems pale diffuse red, (5) flowers smaller (petals $8.12 \times 8-10$ mm.), (6) fruits short, $23-28 \times 5.7$ mm. It might be treated as a separate variety or even species, but it clearly falls into the coastal series which stretches from the south coast of Nova Scotia up the estuary of the St. Lawrence.

The original culture 27.33 contained one aberrant plant, which may be called mut. *hebetifolia*, whose open-pollinated seeds produced culture 50.34. This plant differed from the type in being smaller, with blunt leaf tips and very short fruits. The offspring were of the two types, showing respectively blunt and pointed leaves in the ratio 13:27. Fig. 42 shows a rosette (cf. fig. 40), and fig. 43 the flowering stage (cf. fig. 41). The blunt-leaved type was also later in flowering and the leaves are more or less blotched with pale green. One of these plants, which survived the winter as a rosette and flowered in 1935, had extremely small flowers, rounded, yellowish-green buds, petals only 6 mm. long, ovary 7-8 mm., sepal tips 1-2 mm.,

slender, sub-terminal, green tipped with red. Culture 71.35 was derived by selfing a pointed-leaved plant of 50.34. The offspring were all identical with 70.35.

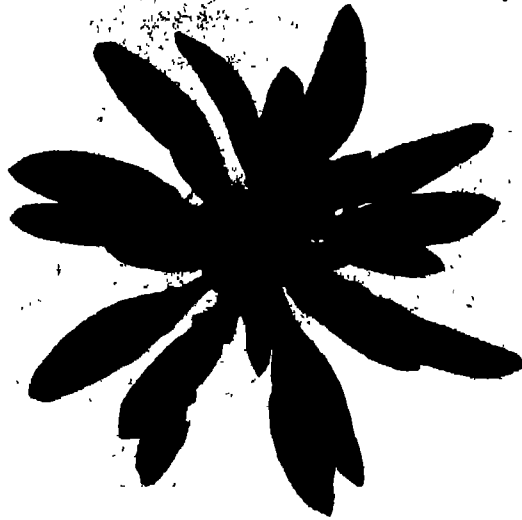


FIG. 42—*O. parva* mut. *hebetifolia*, rosette, culture 50.34.



FIG. 43—*O. parva* mut. *hebetifolia*, in flower, culture 50.34.

Hence the mut. *hebetifolia* segregates the type, which later breeds true. This behaviour is characteristic of a trisomic, but its cytology has not yet been investigated. A plant of the blunt type in culture 50.34 was selfed, but its seeds failed to germinate.

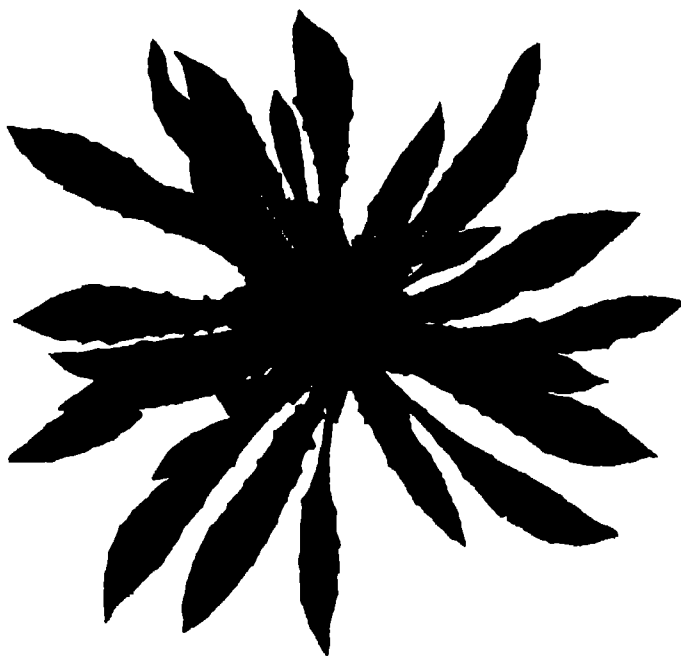
O. leucophylla n. sp.

From seeds collected at St. Valier, Bellechasse Co., Quebec, on 30 September, 1932.

This extremely uniform and distinct species has been studied in the cultures shown in Table XV, all of which were derived from St. Valier, except cultures

TABLE XV

1933	33 (50 plants)	36 (50)	42 (50)			
1934	56 (3)	59 (13)	65 (28)			
1935			76 (33)	133 (31)	134 (31)	
			135 (26)	136 (32)	137 (25)	

FIG. 44—*O. leucophylla*, rosette, culture 59.34.

133-137, which were from seeds collected by Miss MARIE ROUSSEAU at Berthier-en-bas, Montmagny Co., Quebec, on 1 September, 1934.

Description—Rosette leaves small, light pale green, narrowly oblanceolate or elliptic-oblanceolate, apex acute or obtuse, reaching 11-18 cm. \times 23-32 mm. (petiole 3-5 cm.), flat, smooth, or somewhat crinkled, margin repand-dentate or subpinnatifid below, repand-denticulate above, midrib white, finely-appressed pubescence on both surfaces (fig. 44).

Apical 8–10 cm. of stem strongly bent, height *ca.* 60–75 cm., broadly, thickly ribbed throughout, very pale green, with copious red papillae, sparsely ascending- or patulous-hirsute from red papillae and densely finely appressed-crispate-puberulous; sometimes forming basal branches bent at tips, but more usually many short, widely spreading cauline branches, especially from upper part of stem.

Stem leaves usually arcuate-spreading, very narrowly elliptic-lanceolate, wavy, somewhat crinkled, pale green, margin pinnatifid or sub-pinnatifid near base, then much repand-dentate, then repand-denticulate, with numerous green teeth (glands), 8–17 cm. \times 12–32 mm., midrib white above and below, surface appressed or subappressed-pubescent, midrib below pubescent and puberulous in two series. Lower bracts spreading, convex, narrowly lanceolate, *ca.* 5 cm. \times 10–12 mm., upper bracts arcuate-ascending, 1.5–2 cm. long.

Apex of inflorescence flat or convex, conspicuously comose, easily overtopping highest developed bud cones and flowers. Ovary 10–12 \times 2.5 mm., ascending-hirsute from dense red papillae and densely shortly spreading glandular-pubescent. Hypanthium *ca.* 26 \times 2 mm., sparsely patulous hirsute from red papilla (papillae forming streaks) and rather densely spreading glandular-pubescent. Bud-cone dark red (from papillae) where exposed to light, \pm quadrangular, 13 \times 4–5 mm., indumentum as on ovary, sepal tips 4–6 mm. long, divergent, reddish at or near apex. Petal *ca.* 8–10 \times 9–11 mm., not contiguous, opening to 45°, never wide-spreading, widely shallowly emarginate and toothed in sinus. Filaments *ca.* 11 mm., anthers 6 mm., overtopping stigmas by 1–3 mm., base of stigma *ca.* 4–7 mm. above hypanthium, stigma lobes 4–6 mm. long, not or slightly separating (fig. 45).

Diagnosis—Folia radicalia parva, pallida albobiridia, anguste oblanceolata ad elliptico-lanceolata, acuta vel obtusa, plana aut aliquantulum bullata, costa alba. Apex caulis valde declinatus, caulis pallidissime viridis, copiosus rubropapillatus, multis ramis caulinis. Folio caulina angustissime elliptico-lanceolata, undulata, aliquantum bullata, arcuato-extensa. Apex inflorescentiae manifesto comosus. Ovarium dense hirsutum, rubropapillatum, hypanthium rubrolineatum; alabastra papillata, atrorubra ubi lucem accipiunt. Petala circa 8–10 mm. longa.

This species is easily characterized by the small, pale green, rather narrow stem leaves and rosette leaves, and the red papillae on the sepals where exposed to light. The latter character relates the species to *O. ammophiloides*, but it differs markedly from that species in the smaller pale rosettes, in foliage, and in the smaller size of the flowers. *O. leucophylla* also resembles *O. eriensis* in having narrow leaves; but they are pale green, not grey-green and the rosettes are semi-persistent; the flowers are of approximately the same size, but the light-sensitive red papillae of the buds are like those of *O. ammophiloides* and are very different from those of *O. eriensis*, which also differs in having no red papillae on its stems and in dropping its early buds. *O. leucophylla* differs from *O. muricata* L. markedly in the more strongly bent stem tips and in having red papillae on the sepals, as well as in the narrower pale green leaves. It is perhaps most nearly related to *O. eriensis* among earlier species. It has

some features of *O. eriensis* among earlier species. It has some features of *O. eriensis* and of *O. ammophiloides* and it stands between them in its geographical distribution.

No differences were discovered between the cultures from St. Valier and those from Berthier-en-bas except that the latter were distinctly larger, those from St. Valier (76.35) having a height of *ca.* 76 cm. while from Berthier-en-bas ranged

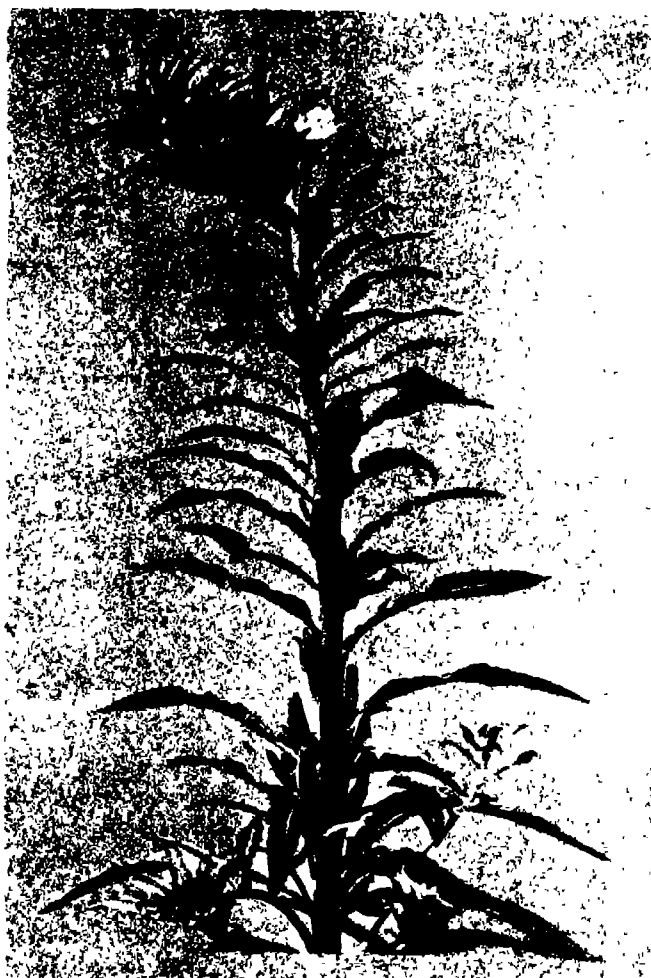


FIG. 45—*O. leucophylla*, in flower, culture 36.33.

from 102–110 cm. Plants from the three original cultures, 33.33, 36.33, and 42.33, from St. Valier, were examined by Mr. FORD and found to have a ring of 14 chromosomes in each case.

O. biformiflora n. sp.

From seeds collected at St. Valier, Quebec, by Messrs. VICTORIN and ROUSSEAU, 1 October, 1932, and myself at Charny, Que., on 3 October, 1932. The large colony

at St. Valier is now known to contain several well-marked species. The colony at Charny was on both sides of the road at the south end of the Quebec bridge. The 17 cultures grown of this species are shown in Table XVI.

TABLE XVI

	St. Valier (cruciate)	St. Valier (broad petals)	Charny (cruciate)			Charny (broad)	St. Antoine les Fonds (broad and cruciate)	
1933	37 (50 plants)	38 (50)	47 (50)			46 (50)	48 (50)	
1934	60 (15)	61 (4)	70 (8)	71 (14)		68 (4)	69 (6)	72 (10)
1935	73 (14)	74 (7)	81 (8)	34 (5)	35 (10)	79 (2)	80 (3)	

The same species has been obtained from all three localities in both the broad-petalled and the cruciate form. The inheritance of this difference will be recorded below.

Description—Rosette leaves dull green, early leaves elliptic or obovate-elliptic, apex obtuse, later leaves oblanceolate, acute or shortly cuspidate, rarely subobtuse, blade reaching 10–36 cm. \times 35–67 mm., petiole 4–5 cm., slightly concave, often conspicuously crinkled near base, midrib pale pink or nearly white, margin repand-dentate below, repand-denticulate above, teeth green, surface rather sparsely appressed-pubescent above, subappressed-pubescent below (fig. 46).

Stem erect, ca. 60–90 cm., basal branches decumbent at base or simply ascending, shorter than central stem which is broadly ribbed, \pm red below, green in upper part, densely patulous-hirsute from conspicuous dark red or colourless papillae and arcuate-subappressed puberulous. Stem leaves spreading or deflexed, \pm concave, middle elliptic, lower elliptic-oblanceolate, upper ovate-lanceolate, acute with purple apex, 7.5–12 cm. \times 29–32 mm., midrib white or faintly tinted with pink in lower leaves. Lower bracts ovate-lanceolate or lanceolate, deflexed or arcuate-deflexed, 4.5–6 cm. \times 19–26 mm. Upper bracts arcuate-spreading or ascending, 15–20 \times 4–7 mm. Apex of inflorescence flattish, slightly depressed, less than 1 cm. across, easily overtopped by developed buds, spike dense. Ovary 8–10 \times 2–3 mm., rather sparsely patulous or ascending hirsute with few small red papillae, and densely shortly glandular-spreading-pubescent. Hypanthium 16–29 \times 1.7–2 mm., very sparsely patulous-hirsute, rather densely glandular-pubescent. Bud-cone greenish or yellowish, squarish, 9–13 \times 4–5 mm., scarcely tapering, sparsely patulous-hirsute, densely glandular-pubescent, no red papillae on bud visible to naked eye. Sepal tips green, 1–2 mm., very slender, terminal, appressed. Petals 12–15 \times 12–23 mm. (cruciate, 12–14 \times 3–5 mm.), broad (or roughly linear with blunt apex), yellow (cruciate petals irregularly marked with green), (fig. 48),

spreading. Filaments 7 mm., anthers 5-6 mm., about level with summit of stigma, lobes 3-5 mm., appressed or spreading, base of stigmas *ca.* 3-5 mm. above hypanthium. Fruits green, 30 × 7 mm., tapered only at apex, surface rough with tuberculations, no red papillae, few short hairs (fig. 47).

Diagnosis—Folia radicalia surda-viridia, obovato-elliptica ad oblanceolata, obtuse ad acuta, costa pallida rubicunda ad paene alba, dentibus margine viridibus. Caulis erectus, folia caulina elliptica ad ovato-lanceolata, acuta, apicibus purpureis,

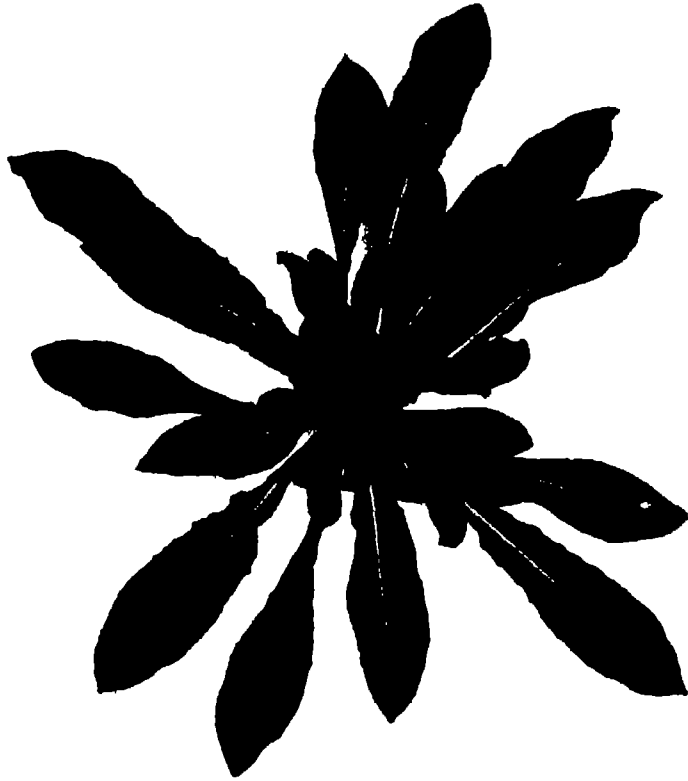


FIG. 46—*O. biformiflora*, rosette, culture 71.34.

costa alba aut languide rubicunda colorata. Ovarium 8-10 mm. longum, hirsutum, paucis parvis rubris papillis; alabastra vix attenuata, viridescens vel flavescens, apices sepalorum 1-2 mm., gracilimi, terminales, appressi, virides. Petala circa 12-15 mm. longa, lata vel cruciata.

This species has both broad-petalled and cruciate plants in all areas where it has been found, but no intermediates appeared except in artificial crosses between the two types. The form with narrow petals may be known as var. *cruciata* n. var. In culture 37.33, the 38 plants which flowered all had cruciate petals. Culture 38.33 had only broad petals, *ca.* 15 × 23 mm., notched, slightly overlapping at base. It

also differed in having (1) white midribs, (2) white papillae on stem and ovaries, (3) rosette leaves strongly repand-dentate to pinnatifid below, with a few purplish blotches. Each of these characters may be controlled by a single gene. The pubescence was also erect rather than subappressed. The same differences could be observed between their descendant cultures 73.35 and 74.35.

Culture 38.33 included one plant which was not observed to differ phenotypically from the rest, but which was shown by Mr. C. E. FORD to be triploid, having 21



FIG. 47—*O. biformiflora* var. *cruciata*, in flower, culture 71.34

chromosomes. The plants from Charny in culture 46.33 all had broad petals, but no red papillae on the stem. This culture was derived from the seeds of four wild plants but was uniform except for one plant, which evidently belonged to a different species. It showed some resemblances to *O. novae-scotiae*, having a red stem, and was selfed to produce culture 69.34. This proved to be a distinct type having pale green, narrowly elliptic or oblanceolate rosette leaves, apex acute or obtuse and apiculate, reaching 18 cm. \times 38 mm., but its flowering stage has not yet been described. In culture 47.33 the single wild parent plant from which seeds were

taken probably had cruciate flowers, since the buds were noted as peculiar, giving the appearance of having been touched with frost, but in the first season only ten offspring flowered, 7 with broad petals and 3 cruciate. One row of 22 rosettes were wintered over and flowered in 1934. The flowers were uniform, markedly smaller than in the St. Valier plants, broad petals uniformly $7-9 \times 7$ mm., obcordate, emarginate. They mostly failed to open, although the plants which were annuals appear to have opened their flowers normally. In the biennial plants the sepals were pushed apart at the base but remained adherent at the apices.



FIG. 48—*O. biformiflora* var. *cruciata*, near view of flowers showing the cruciate character.

A broad-petalled plant gave culture 70.34 with broad petals, measuring 12×12 mm., while a cruciate-flowered sister plant gave culture 71.34, all with cruciate flowers and precisely like 60.34 from St. Valier. Similarly culture 37.33 produced only cruciate offspring in two generations and 38.33 produced only broad petals. Cultures 34.35 and 35.35, both from different broad-petalled plants of 47.33, gave only broad petals. These two cultures were also devoid of red papillae on the stem.

Culture 48.33, from St. Antoine les Fonds, was derived from mingled seeds of two wild plants. Of those which flowered, 25 had cruciate and 8 had broad petals.

They agreed exactly with the other cultures of this series, having midribs and red papillae on the stem. On cruciate plants the petals were not only narrower but considerably longer than on broad-petalled plants. Thus in the latter they measured $7 \times 6-7$ mm., while in the former they were $10-14 \times 3-6$ mm., but in one cruciate plant only 7×2 mm.

Each type thus breeds true when selfed, but seeds of one wild plant gave some broad-petalled and some cruciate-flowered offspring. The broad-petalled and cruciate forms grow intermingled in all three localities. At St. Valier they differ in several features besides the petals, as already pointed out. The broad-petalled strain from Charny differed from the cruciate strain of that locality only in petal width, whereas at St. Antoine the cruciate form had longer as well as narrower petals.

In 1934, reciprocal crosses were made between the broad-petalled and cruciate strains of this species from St. Valier. Culture 60.34 had midribs white or faintly tinged with pink, while in culture 61.35 the midribs were white and the latest rosette leaves were pinnatifid, with basal lobes reaching 7 mm. in length. From cruciate \times broad 18 plants were grown, and 3 plants from the reciprocal. There were no constant differences, the rosettes having faintly pink midribs, later leaves scarcely pinnatifid. In cruciate \times broad the stems bore red papillae, which were absent in the reciprocal. Of the 18 plants from cruciate \times broad, 10 had cruciate flowers, 6 had broad petals, and 2 had petals of intermediate shape or mixed character. The broad petals were *ca.* 17×18 mm., cruciate petals were $12-14 \times 2-4$ mm., while intermediate flowers had petals $15 \times 5-7$ mm. One of the intermediate plants had broad petals on the main stem, intermediate petals on one basal branch, cruciate petals on two other basal branches, and a mixture of petal types on the fourth. The other intermediate plant had both broad and intermediate petals on the main stem, two side branches with intermediate petals, and three with cruciate petals. This behaviour, with both parental types of petal appearing in different F_1 plants, and even in the same plant, as well as intermediate petals, is in accord with earlier studies by DE VRIES (1902) of the inheritance of the cruciate condition. The reciprocal, broad \times cruciate, from the same two parental plants, gave only three offspring, all with broad petals $11-12 \times 14$ mm.

One further cross was made, between two members of the cruciate strain from Charny (71.34×47.33). This cross was made possible by the fact that many plants from the 1933 culture only flowered in 1934. The seed parent had cruciate and the pollen parent broad petals. It yielded 14 uniform plants having the bright red midribs of the Charny strain, 5 of which had cruciate flowers, 5 had broad petals, and 4 were intermediate. The broad petals were 13-14 mm. long. One of the plants had on 6 August broad petals on its main stem, cruciate on 4 basal branches, and broad on one basal branch. The same plants continued flowering and on 17 August had broad petals and one cruciate flower on the main stem, the side branches having some cruciate and some intermediate flowers. Some of the broad-petalled plants also showed later some intermediate flowers. No full explanation of these phenomena need be attempted at the present time.

The absence from all 17 of the cultures of *O. biformiflora* of plants having intermediate petals or a mixture of broad- and narrow-petalled flowers, indicates that natural crossing is rare, or that plants with intermediate or mixed petals are eliminated in nature, each type being stable. There appears to be no difference in size or vigour between plants of the cruciate or latipetalous types, so it appears improbable that hybrids with intermediate petals would be eliminated in nature. On the other hand, experiments to be described below indicate that once a cross is made a mixed and variable condition of the petals continues indefinitely in some of the offspring of later generations.

Cruciate petals furnish one of the most interesting and significant cases of parallel mutations in wild *Oenothera* species. BARTLETT (1914a), in a study of cruciate types, cites the following cruciate forms which are most reasonably interpreted as derived or descended from independent mutations: (1) *O. cruciata* NUTT. described from Massachusetts by DON in 1824, and also apparently found in Vermont; (2) *O. biennis* mut. *cruciata* DE V. = *O. biennis* var. *leptomeres* BARTL., a single plant found at Santpoort, Holland, in 1900; specimens were afterwards obtained from several other localities in Holland, and also from the Lunenburg Heide (KLEBAHN, 1914), and elsewhere in Germany, indicating that the cruciate form arises repeatedly from *O. biennis*;* (3) one branch with cruciate flowers in a culture of an undetermined species from Springfield, Missouri; (4) a form from Mobile, Alabama; (5) *O. atrovirens* SH. and BARTL. from Hudson Falls, N.Y., near Lake George; (7) *O. stenomeres* BARTL. from Maryland, allied to *O. gauroides* HORNEM. from the same area, and practically cleistogamic; (8) *O. stenopetala* BICKN. from Nantucket Id., Massachusetts, related to *O. Oakesiana* (ROBB.) S. WATSON; (9) *O. cleistantha* SH. and BARTL. (1915) from Long Island, N.Y., cultivated by SHULL; (10) *O. Robinsonii* BARTL. (1915) from Jaffrey, New Hampshire. In addition, specimens with cruciate petals in the Gray Herbarium are cited from Nova Scotia (Sable Island), Maine, Cumberland, New Hampshire, Vermont, the Adirondaks, and three localities in Massachusetts. The *O. cruciata* of *Gruppenweise Artbildung* (DE VRIES, 1913) is one of three cruciate forms which DE VRIES cultivated (1913, p. 58). One of these, from Jaffrey, N.H., was afterwards described as *O. Robinsonii* BARTL. The other two came from Hudson Falls, near Lake George, N.Y., via MACDOUGAL and others (1905) (who described some of their characters under the name *O. cruciata*), and differed mainly in the thickness of the buds. The one with thicker buds DE VRIES (1913) used in his later experiments. He found it constant through several generations and used it in many crosses. He briefly characterized it as follows (1913, p. 58): small linear petals, very dense and generally short spike, very small leaves, dark red-brown in nearly all parts, numerous cauline branches, fruits short, young stems strongly nutating. This was described as *O. atrovirens* SH. and BARTL. (BARTLETT, 1913), the other from the same locality as *O. venosa* SH. and BARTL.

* DE VRIES (1913, p. 299) afterwards obtained from it a dwarf mutation, *O. biennis cruciata nanella*, which came true from seed.

The present record shows that *O. biformiflora* occurs commonly in three localities on the south bank of the St. Lawrence in both the cruciate and the broad-petalled form. The large number of records of cruciate forms belonging to various species in eastern North America indicates that this is a mutation which occurs with a high rate of frequency and has arisen independently many times as parallel mutations from different species. Not only is the genus *Oenothera* prone to this mutation, but it has also been described in *Epilobium* (STOMPS, 1913). From a single cruciate plant of *E. hirsutum* found wild in England, he showed by crossing that *E. hirsutum cruciatum* is a simple Mendelian recessive to the type, and that it segregates cleanly without intermediates. OEHLKERS (1935) has recently repeated these crosses, using strains of *E. hirsutum* from three different parts of Germany, and confirming the results of STOMPS. When the crosses were repeated on a larger scale, however, he began to obtain more or less defective, *i.e.*, intermediate, petals.

DE VRIES (1902) originally described the variable behaviour of the petals in *O. biennis cruciata varia*, a strain which he regarded as having arisen in gardens through crosses between *O. cruciata* NUTT. and some other species in Europe. From crosses which he made between it and the three species he recognized as naturalized in Europe, namely, *O. biennis*, *O. muricata*, and *O. Lamarckiana*, DE VRIES concluded that it had been crossed with *O. muricata*. He devoted a section of "Die Mutations-theorie" (II, pp. 593-633) to the study of its hybrids. (This section is omitted from the English translation.) He called *O. cruciata varia* a *Mittelrasse* because of its continuous variability in petal width, but he failed to reach a decision whether this inconstancy had resulted from crossing or from mutation.

The *O. cruciata* of the *Gruppenweise Artbildung* (*vide supra*) agrees in many features with *O. cruciata varia* DE V., and hence probably with *O. cruciata* NUTT. It apparently differs, however, at least in having strongly bent stem tips.

OEHLKERS (1930a, b, 1935) has made an extended study of the variation and inheritance of cruciate petals or sepalody in *Oenothera*. Only a limited number of the results can be referred to here. The forms used in his crosses included *O. biennis cruciata (apetala)* from Leiden Botanical Garden, which was constant, *O. biennis cruciata sulfurea* from Hanover, and *O. Lamarckiana cruciata* from Tübingen Botanical Garden, and *O. biennis cruciata gigas* from Stomps, which were inconstant. Crosses were made with broad-petalled strains of *O. biennis*, *O. suaveolens*, *O. Lamarckiana* and *O. Hookeri*. Among other things it was shown that when broad and cruciate flowers on the same plant are selfed there is a true somatic segregation, since the offspring follow in general the condition of the parent flower. OEHLKERS used the length-breadth index as a measure of the condition of each petal. He concludes that the *cruciata* from *O. Lamarckiana* and from *O. biennis* are both in a labile condition, and postulates a series of *cruciata* genes of different strength, so that in *O. Lamarckiana* a state could be reached in which cruciate was dominant to broad petals. *O. Lamk.* × "weak" *O. Lamk. cruc.* gave 15 plants all with broad petals, while the "strong" *cruciata* produced in F₁ 4 plants with broad petals, 1 with cruciate, and 1 with strongly affected petals. This agrees with results for the

Quebec strains, mentioned above. The F_2 from selfing a strongly cruciate plant contained 27 cruciate and 6 normal, while the F_2 from a normal contained 20 normal, 2 cruciate, and 2 subcruciate. Back crosses were also made. The reciprocal cross *Lamk. cruc.* \times *Lamk.* gave 11 with normal, 2 with cruciate petals, and 2 with strongly affected petals. The conclusion is reached that both the *velans* and the *gaudens* complex of *O. Lamarckiana* contain a *cruciata* factor (cr_4 and cr_3 respectively), but the cr_4 is not strong enough to be dominant to Cr (broad petals). The tetraploid *O. biennis gigas cruciata* was also crossed with *O. biennis gigas*, *O. biennis cruciata*, *O. biennis*, and other forms, the analysis being in terms of the complexes, *albicans* and *rubens*, of *O. biennis*. *O. biennis gigas cruciata* \times *biennis gigas* and the reciprocal gave, in 9 offspring, every condition between purely cruciate and fully normal petals, the plants being otherwise uniform. Both the relative dominance of the cruciate condition and its variability were greater than in the diploid crosses.

It thus appears that many cr genes are present in the *Oenothera* germplasm, and if, for instance, the *velans* and *gaudens* complexes of *O. Lamarckiana* each contain such a factor, then the cruciate mutation may arise through crossing-over between the two complexes, in such fashion that the assemblage of cr genes overbalances the genes making for broad petals. Similarly, the phenotypic expression of the cruciate mutation may only be reached in other species when Cr genes already present are rearranged so that in the zygote they dominate the Cr genes. To determine whether this is an adequate interpretation of the various cruciate mutations and their inheritance requires still further investigations. The fact that there is generally no other difference between the cruciate and broad-petalled races of a particular species makes it difficult to believe that crossing-over of the ordinary type can be involved.

The occurrence of broad, narrow, and intermediate petals on the same plant, as well as in different plants of the F_1 in crosses between wide and narrow petals, is similar to the hereditary behaviour as regards longer and shorter petals in certain *Oenothera* crosses (GATES 1917, 1923). Hybrids of *O. biennis* \times *rubricalyx* and its reciprocal were made, *O. biennis* having petals *ca.* 20 mm. and *O. rubricalyx* *ca.* 40 mm. in length. The F_1 were uniform, but they were few in number and larger numbers might have shown variation in flower-size. In F_2 - F_4 , however, three forms of segregation took place; (1) genetic segregation of plants having longer or shorter mean petal-length and various ranges of variability in this feature. That the segregation was genetic was shown by breeding from large-flowered and small-flowered segregates; (2) segregation between larger and smaller flowers on the same plant, some at least of such plants having a bimodal curve of variability; (3) somatic segregation between longer and shorter petals in the same flower. These three types of segregation also occur as regards the cruciate gene, where the results of OEHLKERS indicate that Cr genes are cumulative in their effects. Other results, mostly unpublished, indicate that small-flowered species of *Oenothera* have several dominant cumulative genes for short petals, some of which produce a larger decrement in the petals than others. It is therefore probable that petal-length and

petal-breadth follow the same rather peculiar laws of inheritance, peculiar in combining genetic with various degrees of somatic segregation.

The present species, *O. biformiflora*, appears to be rather nearly related to *O. cruciata* NUTT., which was grown and described by DON in 1824 from Massachusetts, and has probably been grown in European gardens ever since. The original very general description by DON, a photograph of the type specimen at Geneva, and a description of an apparently identical specimen in Herb. Phila. Acad. by BARTLETT (1914a) indicate similarities to *O. biformiflora* in stem colour and pubescence, in the slender hypanthia, and in bud-size, and perhaps shape. The leaves of *O. biformiflora* are, however, markedly wider, approaching the ovate condition, and the sepal tips are terminal and appressed, whereas in *O. cruciata*, according to BARTLETT, they are "distinctly infra-terminal and well separated in the bud". The *O. cruciata varia* of DE VRIES (1902) has very narrow leaves, as shown by his fig. 136, p. 603. This may have been accentuated by crossing with *O. muricata* as DE VRIES suggests has taken place, but *O. cruciata varia* could not have derived the brown-red colour on the stem-leaves, sepals, and fruits from this source. These characters, together with its short and slender stem, small narrow leaves, small flowers (petals $12 \times 2-4$ mm.), and fruits longer and thinner than *O. biennis*, probably belonged to the original *O. cruciata* NUTT. Since *O. biformiflora* has greenish or yellowish buds and green fruits of good size, these are additional distinctions from the brown-red sepals and (small) fruits of *O. cruciata varia* and hence apparently of *O. cruciata* NUTT. How close the relationship between the latter and *O. biformiflora* may be can only be determined by obtaining *O. cruciata* afresh from Massachusetts, or Vermont. At present it appears preferable to regard them as distinct, especially as *O. biformiflora* is the only species yet known to occur wild with both broad-petalled and cruciate plants intermingled in the same localities. Since *O. stenomeris* BARTL. is allied to *O. gauroides* HORNEM., and *O. stenopetala* BICKN. is similarly related to *O. Oakesiana* S. WATS., each pair has probably diverged from a common ancestor in which the cruciate mutation appeared, followed by an accumulation of other mutational differences under conditions of inbreeding combined with physiological or geographical isolation.

O. laevigata BARTL. var. *similis* n. var.

From seeds collected at St. Valier, at the mouth of the River Boyer, on the south shore of the St. Lawrence, Quebec, on 31 September, 1932, by Messrs. VICTORIN and ROUSSEAU and on 2 October, 1932, by myself. The plant which supplied the seeds for culture 28.33 was noted as tall with bunched fruits, and was preserved by Professor VICTORIN as specimen A in his herbarium. The parent plant of culture 29.33 (specimen B) resembled *O. angustissima* in its long subterminal, spreading sepal tips, red inside, the stem top being dark red with much red on the bracts below. The parent plant of culture 31 was observed to have much red on the under surface of the bracts and fruits with a rather broad attachment. In 1933 the cultures put out long side-shoots, many of which flowered. The plants grown in 1934 from

selfed seeds produced a few flowering side-shoots but wintered over as rosettes and formed tall flowering stems in 1935. An account of the extensive and remarkable *Oenothera* colonies at St. Valier will be found elsewhere in this paper. The cultures of this variety grown are shown in Table XVII.

TABLE XVII

1933	28 (100 plants)	29 (50)	31 (50)	34 (50)	44 (10)	45 (50)
1934	<u>51</u> (46)	<u>52</u> (45)	<u>54</u> (48)	<u>57</u> (49)		

The description here given is fuller than that of BARTLETT (1914*b*).



FIG. 49—*O. laevigata* var. *similis*, rosette, culture 28.33.

Description—Rosette leaves dull green, *ca.* 16–20, oblanceolate to lanceolate, apex acute or shortly cuspidate, reaching 24–39 cm. \times 55–70 mm. (petiole 3–5 cm. long), flattish, with sparse liver-coloured spots on a few leaves of each rosette, crinkled only when young, young leaves conspicuously undulate below and very pale reddish at base, margin subentire or repand-denticulate with obscure reddish teeth; midrib white; upper surface finely sparsely appressed-pubescent and minutely puberulous; lower surface glabrescent to the naked eye, very sparsely appressed-pubescent and very minutely puberulous (fig. 49); rosettes very persistent, forming very long, decumbent, and widely ascending side branches or a central stem generally leaning from the base and strongly bent at the tip.

Stem tall, 138–143 cm., very brittle, tip strongly bent, very leafy, with numerous ascending cauline branches, very strongly ribbed, pale green and deep peach bloom red, rather sparsely suberect or ascending-hirsute from small greenish or reddish papillae, some in the form of red patches with roughened surface, otherwise practically glabrous. Stem leaves deflexed or arcuate-deflexed, elliptic-lanceolate or elliptic-ob lanceolate, somewhat crinkled, margin not wavy, denticulate, sometimes dentate below with 1–2 teeth, 12–17 cm. \times 24–50 mm.; upper surface \pm flecked and margined with red-purple, glabrescent to the naked eye, very finely subappressed-puberulous and scurfy-puberulent, midrib white; lower surface glabrescent, sparsely suberect-puberulous.

Inflorescence dense, not elongating much in fruit; lower bracts spreading, leafy, ca. 5–7 cm. \times 15–22 mm.; upper bracts arcuate-spreading, narrowly oblong-lanceolate, ca. 1–2 cm. \times 24–40 mm., red at base, terminal bracts very small, red on lower surface. Apex of inflorescence bent, narrow, comose, usually tinged with red. Ovary 7–13 \times 2 mm., green or with small red protuberances without a hair, glabrous or very sparsely spreading glandular pubescent in upper half; hypanthium 27–37 \times 1.5–2 mm., pale green, glabrous; bud-cone 11–16 \times 4–5 mm., sharply quadrangular, greenish-yellow, or \pm reddish, especially on shoulder and base, slightly tapering, sepal tips rigid, erect, slender, subterminal, hooded within, scarcely divergent, 3–4 mm., green with reddish tips and a touch of red at the base, not densely but distinctly ascending pubescent and puberulous. Petals 10–15 \times 11–16 mm., truncate or shallowly widely emarginate, opening to 45°, slightly overlapping. Filaments ca. 7 mm., anthers 5–6 mm. Stigma lobes 1–4 mm., surrounded by anthers in bud; in anthesis the lobes become slightly divergent, but owing to elongation of the hypanthium, they are drawn down nearly or quite into the mouth of the tube although occasionally they may be as much as 6 mm. above. Fruits green, glabrous, tapering, 36 \times 7 mm., \pm curved, according to position of stem (fig. 50). Plants from the three cultures, 28.33, 29.33, and 31.33, were found by Mr. FORD to have a ring of 14 chromosomes in each case.

Diagnosis—*Folia radicalia surda viridia, oblanceolata ad lanceolata, acuta aut breviter cuspidata, subplana, sparse purpureomaculata; costa alba; dentes marginales rubescentes; rosulae valde permanentes instructae ramis radicalibus longis aut caule medio declinante, apex valde flexus. Caulis et rami radicales foliosi cum ramis caulinis ascendentibus, pallidis viridibus et atrorubris. Folia caulina elliptico-lanceolata, marmorata et marginata purpura, oculis nudis subglabra. Inflorescentia densa in fructus, apex angustus, comosus, rubrotinctus. Apices sepalorum rigidi, erecto tenuiter, subterminales. Petala 10–15 mm. longa, stigmata 1–4 mm. longa, ad aut prope os hypanthii in floribus apertis.*

In culture 29.33 the petals reached 17 \times 20 mm., and remained slightly larger in the F_1 . The position of the stigma varies greatly in this species. In one plant of culture 34.33 the stigma lobes in all the flowers were “drawn down” some distance into the hypanthium tube by its growth, the lobes being only 1 mm. in length. They

were already pollinated, as the anthers surround the stigma in the bud and are ruptured the day before the flower opens. In some plants of this culture the stigmas reached above the top of the anthers in bud and were just above the mouth of the hypanthium in flower. The hypanthium increases in length during the intervening period, from *ca.* 26 mm. to 30–34 mm. The stigmas in the open flower may be as much as one-third of the way down the length of the hypanthium tube, and they may even be in the tube at the bud stage, so that self-pollination cannot take place and cross-pollination is also made almost impossible.



FIG. 50—*O. laevigata* var. *similis*, in flower, culture 28.33.

BARTLETT (1914*b*) described *O. laevigata* from White Sulphur Springs, W. Virginia, having the striking feature that the rapid elongation of the hypanthium pulls the stigma down into its mouth. He states that allies of this species are widespread in the Alleghanian region. Var. *similis* is evidently a still more northerly representative of the same species, occurring on the St. Lawrence. These strands of descent, as it were, running north and south, probably represent a line of migration northwards following the retreat of the ice. On the Pacific coast a similar line representing *O. Hookeri* TORR. and GRAY, and its descendants, can be traced from California into British Columbia.

Var. *similis* agrees with *O. laevigata* especially (1) in being glabrescent, and (2) in having the stigma drawn down into the hypanthium in anthesis, (3) quadrangular buds. There are, however, a number of differences in var. *similis*, which has (1) wider, dull green rosette leaves with sparse liver-coloured spots and white midribs, (2) somewhat smaller flowers, (3) inflorescence dense, (4) the drawing down of the stigma appears to be more extreme. It may be that a fuller comparison of these forms will result in raising the variety to specific rank, but it appears preferable to recognize the close relationship by retaining the St. Lawrence form as a variety.



FIG. 51—*O. laevigata* var. *rubripunctata*, rosette, culture 53.34.

O. laevigata BARTL. var. *rubripunctata* n. var.

From seeds collected at St. Valier, Quebec, on 30 September, 1932, by Professor VICTORIN and M. ROUSSEAU, and by myself on 2 October, 1932, from the same colony. The wild plant from which seeds for culture 30.33 were taken showed resemblances to *O. ammophiloides*, having a leaning stem, large fruits bending upwards, and red blotches. It was pressed by Professor VICTORIN as specimen E. The cultures grown are shown in Table XVIII.

TABLE XVIII

1933	30 (50 plants)	32 (50)	35 (50)
1934	53 (49)	55 (50)	58 (30)

This strain is nearly related to *O. laevigata* var. *similis* from the same locality, but differs markedly and constantly in several features. It also shows marked similarity to *O. ammophiloides* var. *laurensis* from Port Elgin, and Cape Tormentine, N.B., especially in the leaning or strongly bent stems and the hairy buds with numerous red papillae on the exposed side of the sepals. It differs from *O. laevigata* var. *similis* in (1) smaller, narrower, smooth rosette leaves, duller green, with no red



FIG. 52—*O. laevigata* var. *rubripunctata*, in flower, culture 30.33.

spots (fig. 51), reaching 29 cm. \times 42 mm., (2) ovary covered with red papillae bearing long hairs, (3) bud-cone studded with conspicuous red papillae where exposed to light, (4) stigmas from just above to well above hypanthium, less variable in position, (5) fruits large, 40 \times 10 mm., tapering, straight or curved at the tip (fig. 52). A plant of culture 30.33 was found by Mr. Ford to have a ring of 14 chromosomes.

This strain shows remarkable relationships to the glabrate *O. laevigata* from W. Virginia, on the one hand, and to the hairy budded *O. ammophiloides* from Nova

Scotia on the other. It should perhaps be raised to specific rank, but for its striking resemblances in habit and foliage to *O. laevigata* var. *similis*.

Diagnosis—A varietate simili sic differt : folia radicalia angustiora, surdiora viridia, non purpureo-maculata. Ovarium multirubropapillatum, sepala etiam rubropunctata ubi lucem accipiunt, stigmata supra hypanthium ; fructus magni, 40 mm. longi, 10 mm. lata ad basim, attenuati.

Differs from *O. laevigata* and var. *similis* in (1) narrower rosette leaves, duller



FIG. 53—*O. laevigata* between var. *similis* and var. *rubripunctata*, rosette, culture 62.34.

green, with no red spots, (2) ovary red punctate, (3) bud cone red punctate where exposed, (4) stigmas above hypanthium.

The cultures shown in Table XIX represent a strain from St. Valier which combines features of var. *similis* and var. *rubripunctata*.

TABLE XIX

1933	39 (50 plants)	
1934	62 (47)	63 (18)
1935	75 (34)	

It may represent a hybrid between them. The rosettes were, however, narrower-leaved than either (fig. 53), they were less grey-green than var. *rubripunctata*, and nearly glabrous like var. *similis*. The stem tips were strongly bent and bore a few red papillae (fig. 54); the buds had many red papillae, as in *rubripunctata*, petals 18×24 mm., hypanthium 41×2.5 mm., arcuate, stigma above the tube in anthesis. This condition was, however, not constant, because plant I.1 (the parent of culture 62.34) had so short a style that in certain open flowers the stigma reached only half-way up the hypanthium tube, the stigma being very tiny. In late flowers of



FIG. 54—*O. laevigata* between var. *similis* and var. *rubripunctata*, in flower, culture 62.34.

this plant the style appears to have aborted and dried up. Cultures 62.34 and 63.34, however, showed no differences at all, both breeding true to the conditions above described for this strain, and retaining certain features of both varieties. Mr. C. E. FORD examined a plant in culture 39.33 and found a ring of 14 chromosomes.

O. Victorini GATES and CATCHESIDE

This species, described from St. Hubert near Montreal (1933), is nearly related to *O. pycnocarpa* ATK. and BARTL. which is widespread in New York State. *O. Victorini* is found roughly from the St. Lawrence below Quebec to Montreal and Toronto.

Several varieties of both species are here described. The two species differ mainly as shown in Table XX.

TABLE XX

	<i>O. pycnocarpa</i>	<i>O. Victorini</i>
Rosette leaves	Smooth or \pm crinkled, oblanceolate	Smooth, elliptical.
Upper rosette leaves . . .	Deeply pinnatifid	Not pinnatifid.
Stem	Red papillae on stem	Green papillae on stem.
Petals	16-20 mm.	26 \times 30 mm.
Fruits	25-33 \times 5 mm.	Reaching 45 \times 7 mm.

O. Victorini may therefore be looked upon as a more northern ally of *O. pycnocarpa*, having differences in leaf-shape, especially in the absence of pinnatifid lobing, absence of red papillae from the stem, larger flowers and fruits. They agree in general habit, and in having petals which are firm and resistant to wilting, but *O. pycnocarpa* is generally a more luxuriant plant with more basal branches. Both species cover a wide area and show several varieties. The following three varieties of *O. Victorini* are here described.

In 1932 and the following year cultures were grown from seeds collected at West Wittering, Sussex, where an *Oenothera* is naturalized. They proved to be the same as *O. Victorini*, differing from the type cultures only in having a somewhat shorter stem bearing red papillae. How the introduction took place is quite unknown, but it was presumably unintentional, and probably from somewhere in the area between Montreal and Quebec.

O. Victorini var. *parviflora* n. var.

Seeds sent by Professor MARIE-VICTORIN were collected on 12 October, 1931, from four different plants among a colony growing on dry gravelly or sandy soil on waste ground at St. Anne, Kamouraska Co., Quebec. The resulting cultures (Table XXI) were uniform, except for slight variation in petal length. The cultures from St. Antoine les Fonds differ in having slightly larger petals (13-14 \times 12 mm.).

TABLE XXI

	St. Anne, Kam. Co.				St. Antoine
1933	56 (40 plants)	57 (50)	58 (22)	59 (50)	49 (13)
1934	80 (5)	81 (3)	82 (11)	83 (4)	73 (4)
1935	88 (6)	89 (11)	90 (6)		82 (8)

Description—Rosette leaves pale green, elliptic, obovate-elliptic or oblanceolate, apex shortly cuspidate or acute, reaching 24 cm. \times 55 mm. (petiole 4-5 cm.), flattish and smooth, midrib whitish with pinkish tinge, margin repand-dentate or denticulate below, repand-denticulate above, teeth green, both surfaces finely rather sparsely \pm erect-pubescent, also densely minutely crispulous-appressed-pubescent on midrib, leaves with liver-coloured blotches.

Stem erect, *ca.* 83 cm., basal branches decumbent at base, then widely arcuate-ascending, shorter than central stem. Stem rather thickly ribbed throughout, pale green, sparsely patulous-hirsute with white papillae, arcuate-ascending-pubescent and subappressed-crispate-puberulous. Stem leaves deflexed or uppermost spreading, elliptic-lanceolate (uppermost lanceolate), 11–15.5 cm. \times 32–49 mm., flattish, not wavy or crinkled, margin repand-dentate below, repand-denticulate above, teeth usually green, midrib nearly white but faintly pinkish-mauve in lower half, \pm appressed-puberulous and with some longer hairs below. Both surfaces rather sparsely suberect-pubescent. Lower bracts arcuate-spreading, concave, 4.5 cm. \times 12–17 mm. Upper bracts arcuate-spreading, or patulous-ascending with upcurved tips, 1.5–2 cm. \times 3–7 mm. Apex of inflorescence narrow (*ca.* 1 cm.), flat, not comose, easily overtopped by upper developed buds and flowers, spike dense.

Ovary 9–11 \times 2.5–3 mm., sparsely patulous-hirsute from colourless papillae, and densely spreading glandular-pubescent. Hypanthium 20–26 \times 1.7–2.2 mm., very sparsely patulous-ascending-hirsute and rather sparsely glandular-pubescent. Bud-cone \pm cylindric, pale green, 11 \times 5 mm., extremely sparsely patulous-hirsute from colourless papillae and rather densely spreading glandular-pubescent. Sepal tips *ca.* 2 mm., slightly divergent and reddish. Petals 7–11 \times 6–10 mm., truncate and irregularly toothed, opening to 45°, not overlapping. Filaments 7–9 mm., anthers 3.5–5 mm., overtopping stigmas in bud, base of stigma *ca.* 3 mm. above hypanthium, stigma lobes 4–6 mm., widely divergent, soon arcuate-divaricate.

Diagnosis—A species sic differt: Flores minores, petala 7–14 mm. longa.

This variety differs from the species mainly in having smaller flowers (petals 7–14 mm. instead of 26 mm.). There is also evidence of minor differences in flower-size within this variety, in cultures 80.34 to 83.34. Another collection of seeds of *O. Victorini* from the original locality, St. Hubert near Montreal, by Miss MARCELLE SAUVREAU on 19 September, 1931, differs from the type (*see* GATES, 1933, p. 182) in having petals of intermediate size (11–14 mm. long), as shown by cultures 55.33, 79.34, and 87.35. The variety *parviflora* therefore exists with the type at St. Hubert. From much statistical work it is shown that in some species of *Oenothera* a series of genes for petal-size are present, but the range in *O. Victorini* (7–26 mm.) is exceptionally wide. Observations show that in *O. novae-scotiae* there is a large-flowered segregate, so that the range is 15–26 mm. The St. Antoine strain agrees with *O. Victorini* in having no red papillae on the stem, but it differs in having white midribs.

O. Victorini var. *intermedia* n. var.

Three lots of seeds have been collected which belong to this variety: (1) from St. Valier on 30 September, 1932, by Professor VICTORIN and M. ROUSSEAU. (2) from the railway embankment near Ste. Anne de Bellevue, Jacques Cartier Co., Que., 5 November, 1933, by Mr. W. G. DORE, received through the courtesy of Dr. R. INGALLS, (3) from Cap Tourmente, Montmorency Co., Que., on 12 October, 1933,

by Messrs F. MICHEL and M. L. CHOLLET. The cultures grown are shown in Table XXII.

TABLE XXII

	St. Valier	Ste. Anne de Bellevue			Cap Tourmente	
1933	43 (50 plants)					
1934	66 (7)	4 (5)	5 (97)	6 (98)	9 (7)	10 (4)
1935	77 (27)		40 (9)	41 (17)	42 (26)	45 (21)

A plant culture 43.33, examined by Mr. C. E. FORD, showed a ring of 14 chromosomes.

This variety of *O. Victorini* from both shores of the St. Lawrence is intermediate in flower-size between the species and its var. *parviflora*. The rosette leaves agree with var. *parviflora*, but the stem leaves are narrower (8.5–18 cm. \times 17–34 mm.). The petals are 15–19 \times 11–17 mm., the upper half spreading flat in anthesis (*see* fig. 55). The plants continue flowering very late, producing numerous flowers from cauline branches at the end of the season. The Cap Tourmente strain differs in certain particulars, having narrower rosette leaves (25–40 mm. wide) with finely appressed pubescence. The petals remain stiffly erect, but the plants have the same habit of producing numerous late flowers from cauline branches. In culture 45.35 the midribs ranged from white to pink and all the plants except four had red papillae on their stem, but neither character could be sharply scored.

Diagnosis—A specie sic differt: folia radicalia et caulina angustiora, petala media longitudine, 15–19 mm. longa, diutissime floescentia in ramis caulinis.

O. Victorini var. *undulata* n. var.

From seeds collected on York Mills Road, near Toronto, Ont., 7 October, 1932. While belonging to *O. Victorini* they form a well-marked variety with small flowers, but culture 63.33 and its descendants differ from var. *parviflora* in other characters. The cultures grown and studied are shown in Table XXIII.

TABLE XXIII

1933	62 (100 plants)	63 (50)
1934		85 (13)
1935		91 (16)

Agrees with the St. Anne (Kaimouraska) cultures (*i.e.*, var. *parviflora*) in having small flowers (petals 10–12 mm.) but differs in having leaves with white midribs, \pm crinkled, margin markedly undulate, lower stem leaves broader (12–21 cm. \times

25–60 mm.), deeply pinnatifid at base, margin serrate. The leaves, buds, and stems are also less sparsely pubescent and the teeth green or reddish. The stems are taller (85–95 cm.) and the bracts somewhat smaller. The fruits are short and stout in this variety, 24×7 mm., as against 45×7 mm. in the species. This description applies especially to culture 63.33 and its descendants. Culture 62.33 differed in being almost devoid of the crinkling, undulation, and serration of foliage found in var. *parviflora*. This Eastern Ontario form stands rather apart from the rest of the



FIG. 55—*O. Victorini* var. *intermedia*, in flower, culture 5.34.

species and its relationships will be clearer when other *Oenotheras* from this region have been studied. A ring of 14 chromosomes was found by Mr. C. E. FORD in a plant from culture 63.33.

Diagnosis—A specie sic differt : folia, caules et sepala minus sparse pubescentia ; costa alba ; folia \pm bullata, margo valde undulata, folia caulina inferiora latiora, valde pinnatifida ad basim ; caules altiores, petala 10–12 mm. longa, fractus breves et robusti.

O. angustissima var. *quebecensis* n. var.

From seeds collected at Cap Tourmente, Montmorency Co., Quebec, on the north shore of the St. Lawrence on 12 October, 1933, by Messrs. F. MICHEL and M. L. CHOLLET and sent by Professor MARIE-VICTORIN. The following cultures were grown :—

1934	8 (42 plants)	11 (84)
1935	44 (28)	

O. angustissima was described from Ithaca, N.Y. (GATES, 1913). It stands apart from other species in eastern North America. It has been shown (GATES and

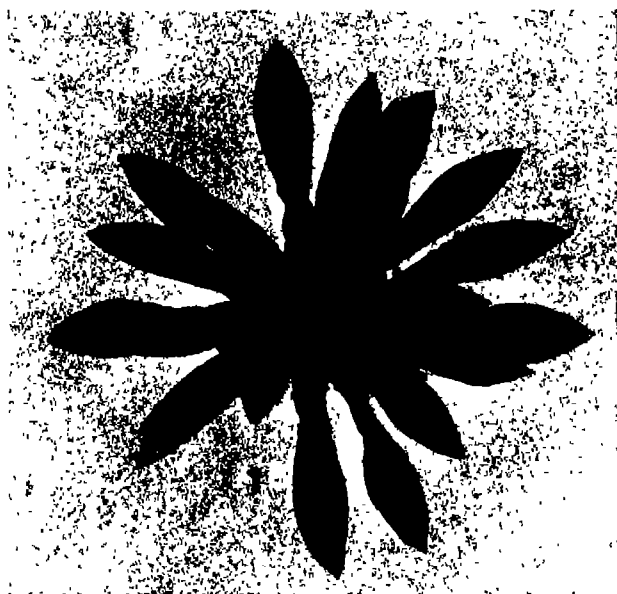


FIG. 56—*O. angustissima* var. *quebecensis*, rosette, culture 11.34.

CATCHESIDE, 1932) to be composed of two distinct complexes, which were called *rubrans* and *divergens*. Its full formula is *divergens* (*rubrans*) ♀. *divergens* ♂, only one pollen complex being functional and the large majority of the megaspores carrying the *divergens* complex owing, apparently, to its greater strength in competition with *rubrans*. These two complexes also differ in flower-size, *rubrans* carrying genes for larger flowers than *divergens*; the latter also has a factor for bent stem tip—a condition which is present phenotypically in var. *quebecensis*.

MARIE-VICTORIN has seen my cultures, and in his "Flore Laurentienne" (1935) recognizes *O. angustissima* as a constituent of the Quebec flora. The present strain from north of the St. Lawrence represents a well-marked variety, but it could belong to no other species and it extends the distribution of *O. angustissima* to a distance of some 400 miles from the originally known locality. It agrees with the

species in all its main characters, being glabrate, with deep red stems and midribs, stem tips nutating, narrow leaves, and markedly subterminal sepal tips. It differs constantly, however, in having (1) wider rosette leaves (3–4 cm. against 25 mm.) and stem leaves (9–16 cm. \times 20–30 mm. against 25 cm. \times 15 mm.), (2) numerous small liver-coloured blotches on the leaves (fig. 56), (3) sepals pale yellow with faint red streaks, (4) ovary and hypanthium becoming red on exposed side, (5) sepals



FIG. 57—*O. angustissima* var. *quebecensis*, in flower, culture 8.34.

bearing many long hairs from colourless papillae, (6) stigma lobes remaining appressed, (7) stem tip slightly bent, (8) fruits very tapering, reaching 40×7 mm. (fig. 57).

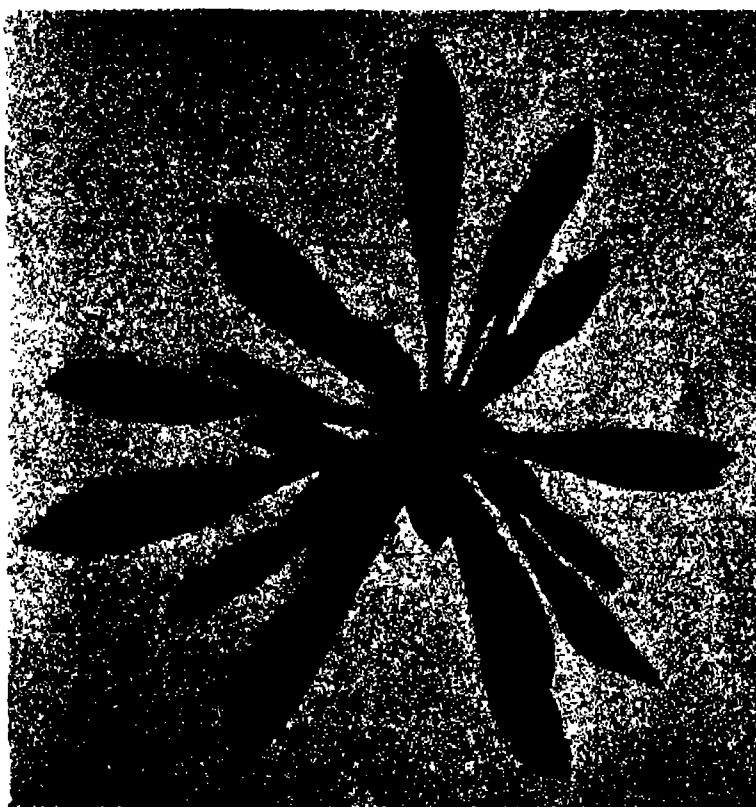
Diagnosis—A specie sic differt : folia latiora cum multis parvis purpureis maculis ; sepala pallida flava, infirme rubrolineata ; ovarium hypanthiumque rubrum ubi lucem accipiunt, sepala multas longas pilas de pellucidis papillis ferentia ; fructus admodum attenuati.

O. niagarensis n. sp.

From seeds collected in the Niagara Gorge, on the American side, 28 August, 1932.

TABLE XXIV

1933	2 (28 plants)		
1934	20 (54)		
1935	47 (5)	48 (7)	49 (11)

FIG. 58—*O. niagarensis*, rosette, culture 47.35.

Description—Rosette leaves dull, somewhat greyish-green, smooth, rather narrowly oblanceolate, apex acute to shortly cuspidate, reaching 25 cm. \times 30–40 mm. (petiole 6–8 cm.), margin repand-dentate to subpinnatifid below, repand-denticulate above, midrib pink or white, both surfaces very finely appressed-pubescent. The young rosette leaves are margined with pink and also have diffuse pale red near the base, making the heart of the rosette brownish red (fig. 58).

Stem ca. 76–94 cm., stout, strongly ribbed, the terminal 4–10 cm. bent horizontal, stem pinkish at base, green above (no red papillae) or with tinges of pink and widely scattered small red papillae. Ring of basal branches, long-decumbent at base

then widely patulous-ascending, some exceeding the central stem ; short cauline branches from the axil of nearly every leaf, widely ascending. Stem sparsely ascending or subappressed hirsute and densely, minutely appressed crispulous-puberulous. Stem leaves mostly deflexed, some of upper spreading, narrowly elliptic-lanceolate, somewhat greyish-green, acute, tips red, reaching 18 cm \times 33 mm., short petiole decurrent as ridge on stem, leaf margin scattered repand-dentate, denticulate near apex, teeth reddish or green ; finely, sparsely subappressed



FIG. 58—*O. niagarensis*, in flower, culture 47.35.

puberulous on both surfaces, base of young leaves tinged with red above. Midrib white, with some long patulous hairs below (a temporary touch of pale red at base of petiole on each side of midrib). Upper bracts heliotropic-spreading on bent apex, red-tipped, apex recurved, 2-3 cm. long (fig. 59).

Inflorescence lax, with long lower internodes, apex convex, very comose, innermost bracts pink with green tips, much longer than upper fully developed buds. Many of the buds drop when very young. Ovary 10-12 mm. \times 2.5 mm., steeply ascending

or subappressed short hirsute with small, mostly green papillae, and subappressed arcuate-pubescent. Hypanthium 23–30 mm. \times 1.5–2 mm., shortly ascending-hirsute and subappressed crispulous-puberulous, reddish on exposed side. Bud cone yellowish, sometimes with streaks of red, squarish, conspicuously attenuate, 12–14 mm. \times 4.5 mm., \pm ascending-hirsute and shortly ascending-arcuate-pubescent. Sepal tips very pointed, 3–4 mm. long, appressed, or spreading from base, green or pinkish in upper half. Petals 9–13 \times 10–12 mm., truncate or rather conspicuously widely emarginate, opening to 45°, with narrow spaces between, at cuneate base. Filaments 7–8 mm. long, anthers 4.5–5 mm. long. Stigma lobes 7–8 mm. long, base ca. 5 mm. above hypanthium, lobes widely separate from the first, reaching 3–4 mm. above anthers. Fruits green, short, and strongly tapering to slender apex, ca. 21 \times 6 mm., frequently somewhat curved, bearing scattered hairs without papillae.

Diagnosis—Folia radicalia surda aliquantum canoviridia, plana, aliquantum anguste oblanceolata, acuta aut breviter cuspidata, costa rubicunda ad alba, folia radicalia nova margine rubicundo, media rosula rubrofusca. Caulis robustus, apex horizontaliter flectens, viridi- et rubicundo-tinctus, non rubro-tuberculatus, rami caulini de axilla omnium foliorum. Bractae superiores extendentes, in apice flexo, rubro acumine apex recurvatus. Inflorescentia laxa, apex convexus, comosis-simus; apices sepalorum 3–4 mm. longi, admodum acuti; appressi vel divergentes de basi; petala circa 9–13 mm. longa. Fructus breves, valde attenuati.

This species is clearly related to *O. eriensis*, with which it agrees in having narrow grey-green foliage, bent stem tips, in flower-size, and in dropping its buds. It differs constantly, however, in having (1) wider leaves, bearing red pigment when young, (2) a well-formed rosette, (3) a stouter stem straight except at tip, whereas *O. eriensis* has a more slender stem more or less irregularly bent, as though the gravitational response was variable. *O. niagarensis* has the habit of *O. ammophiloides* and the foliage is similar, but the midribs may be white or pink (white in *O. ammophiloides*). It differs also in having no red papillae on the stem, much smaller flowers, and much less hairy fruits. It has been constant in cultures. The touch of red which appears on the stem leaves at the base of the petiole on each side of the midrib below, completely disappears later. I classify this as an *evanescent character*. It appears regularly but temporarily on each leaf, and is therefore not a fluctuation but has a genetic basis.

O. repandodentata n. sp.

From seeds collected in the sandy bank on the north shore of Lake Erie, at Colchester, Essex Co., Ont., on 9 October, 1932. This was the original locality for *O. eriensis* (see GATES, 1927, 1928), and on account of the narrow grey-green leaves it was supposed, when collected, to be the same species, although it showed certain differences. The original plant from which seeds were collected was observed, however, to have strongly denticulate bracts, a compact inflorescence, red stripes

on the hypanthium and sepals, and small, narrow, notched petals with wide spaces between them. These are all distinctions from *O. eriensis*. The new species has bred perfectly true for three generations, as shown by the following cultures :

1933	70 (50 plants)
1934	94 (47)
1935	97 (30)

Description—Rosette leaves 14–18, “mat” pale greyish-green, narrowly oblanceolate or elliptic-oblanceolate, apex acute, reaching 16–19 cm. \times 15–25 mm. (petiole

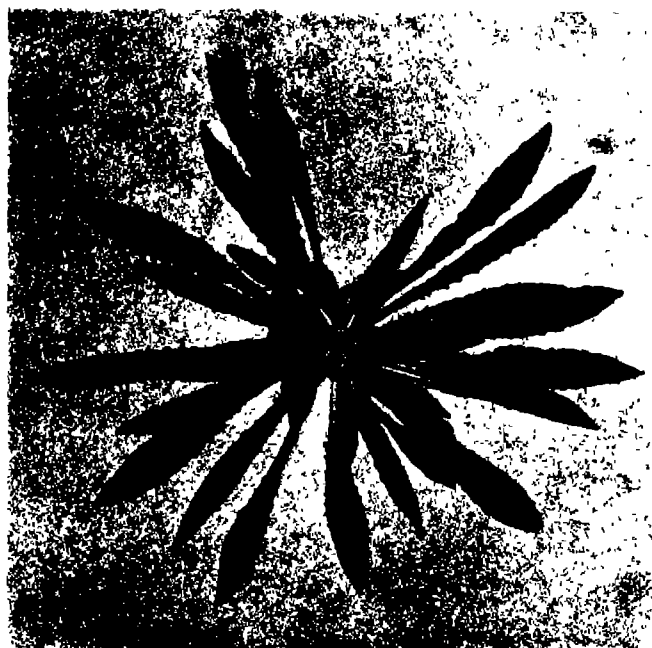


FIG. 60—*O. repandodentata*, rosette, culture 94.34.

5–5.5 cm.), flattish, without crinkling or undulation, margin rather finely and sparsely repand-dentate below, repand-denticulate above, teeth usually red, midrib faintly pinkish-mauve, both surfaces finely rather sparsely appressed-pubescent (fig. 60).

Stem *ca.* 78 cm., erect when growing or slightly bent at extreme tip, basal branches none or (usually) several slightly decumbent, then steeply ascending, shorter than central stem, bent at tip, many cauline branches, long, steeply ascending, \pm bent at tip (fig. 61). Stem strongly and thickly ribbed above, a beautiful deep peach-bloom pink-mauve, except upper third which is greenish; sparsely patulous or ascending-subappressed hirsute from red papillae, and densely minutely appressed crispulous-puberulous; collar green or pink. Stem leaves arcuate-spreading or

lower arcuate-deflexed, elliptic-lanceolate, acute, red tips, convex, not wavy; margin regularly repand-dentate throughout, repand-denticulate only near apex, teeth green or reddish; size *ca.* 12 cm. \times 21-27 mm., midrib faintly pinkish-mauve in lower half and densely appressed-crispulous-puberulous above, also with some longer patulous hairs below, leaf surface subappressed-pubescent above, rather sparsely below. Lower bracts spreading or patulous-ascending, concave and toothed like leaves, lanceolate, 9-10 cm. \times *ca.* 25 mm. Upper bracts arcuate-patulous, crowded, 2-2.5 cm. long, uppermost 5 mm. or less in width. Apex of



FIG. 61—*O. repandodentata*, habit, culture 70.33.

inflorescence depressed, very comose, easily surpassing highest developed bud-cones. Spike with long internodes, not dense, few-flowered (fig. 62).

Ovary 10-13 \times 2.5-3 mm., copiously ascending-hirsute from dark red or colourless papillae and densely crispulous \pm appressed-pubescent. Hypanthium 26-31 \times 2 mm., indumentum as on ovary, but no red papillae and also sparsely spreading-glandular-pubescent. Bud-cone greyish-yellow, squarish, 12-15 \times 5 mm., patulous or ascending sparsely short-hirsute from reddish or colourless papillae, and densely subappressed pubescent. Sepal tips 2-4 mm. long, slender, green or reddish at apex, \pm appressed. Petals opening to 45°, 14-18 \times 11-14 mm., not at all

or slightly overlapping, conspicuously deeply emarginate (sinus 1.5–2 mm.). Filaments 10 mm., anthers *ca.* 6 mm., nearly reaching top of stigmas in bud, stigma lobes 4–9 mm. long, slightly or widely separating. Fruits very stout, 32×9 mm., tapering, green, few long and short hairs.

Diagnosis—Folia radicalia surda pallida canoviridia, anguste oblanceolata vel elliptico-oblanceolata, acuta, plana, margine repando-dentata ad basim. Caulis



FIG. 62—*O. repandodentata*, in flower, culture 94.34.

erectus vel levissime declinatus, rubropapillatus, rubicundo-purpureus, triens supra viridescens, rami valde ascendentes. Folia caulina elliptico-lanceolata, acuta, apicibus rubris, tota margine constanter repando-dentata. Inflorescentia non densa, paucis floribus, apex depressus, multum comosus. Petala 14–18 mm. longa, valde emarginata. Fructus robustissimi et attenuati.

This species, while clearly related to *O. eriensis*, differs markedly in nearly all its characters. The most prominent differences are (1) rosette less evanescent, stem pinkish-mauve, nearly erect, (2) leaves wider, with faintly pinkish midrib, more

ascending and longer, margin regularly repand-dentate, (3) petals larger, with deep sinus. *O. eriensis* has not only bent tips but an irregularly bent stem. In *O. repandodentata* the stem is always erect except the extreme tip, and in some stages of growth or under some conditions, it may be entirely erect.

O. deflexa n. sp.

This very distinct species is common on waste ground in the vicinity of Windsor, Ontario. Seeds were collected from six localities on 9 October, 1932, from which many cultures have been derived. They show numerous minor but marked differences, which are constant in the cultures and will be described below. The cultures with their place of origin are as follows, the specific description being taken from culture 89.34. The plant from which these seeds were taken was on the grounds of the Canada Steel Corporation, and the following characters were noted on adjacent plants: rosettes dark green, leaves crinkled, stems red, flowers small, fruits smooth.

TABLE XXV

	Windsor, Ont.	Ojibway, Ont.	Sandwich, Ont.			Colchester, Ont.
1933	65 (49 plants)	66 (50)	67 (50)	68 (50)	69 (50)	71 (50)
1934	88 (4)	89 (5)	90 (34)	91 (47)		95 (8)
1935	93 (4)	94 (3)	95 (3)	96 (5)		98 (4)

Description—Rosette leaves pale green, narrowly elliptic-oblancoate, apex acute, reaching 19–23 cm. × 32–40 mm. (petiole 4.5–6 cm.), flattish or slightly concave, slightly crinkled, usually undulate, margin repand-dentate below, repand-denticulate above, teeth usually green, midrib white with pinkish tinge, surface finely subappressed-pubescent above, subappressed to suberect pubescent below; rosette stage ± evanescent (fig. 63).

Stem erect, tall, ca. 100–112 cm., basal branches none or short, cauline branches numerous, long and ± arcuate-ascending. Stem weakly ribbed below, strongly ribbed above, exorticating at base, green streaked with red (branches frequently very red in parts), patulous or ascending-hirsute (two series) with reddish or ± colourless papillae and appressed-crispate-puberulous. Stem leaves spreading or arcuate-deflexed, lanceolate or elliptico-lanceolate, acute, red-tipped, ± concave, conspicuously wavy; lower subpinnatifid and strongly repand-dentate below; upper repand-dentate below, repand denticulate above, teeth green, size 11–22 cm. × 25–45 mm.; midrib white or faintly tinged with pinkish mauve, green below with scattered long hairs; surface very sparsely suberect-pubescent and copiously subappressed-puberulous above, subappressed or suberect-pubescent with scattered longer hairs below. Lower bracts spreading or sometimes arcuate-deflexed, very concave and wavy, lanceolate, 6–8 cm. × 20–28 mm. Upper bracts arcuate-deflexed

and very undulate and concave, uppermost deflexed or spreading, flat, not wavy, 13-23 mm. long, red-tipped. Apex of inflorescence flat or slightly depressed, exceeding or level with or slightly exceeded by outer developed bud-cones and flowers (fig. 64).

Ovary 10-11 \times 2-2.5 mm., not densely ascending or patulous hirsute with green papillae, and rather densely spreading-glandular-pubescent. Hypanthium slender, 20-22 \times 1.5 mm., very sparsely hirsute and not densely glandular-pubescent. Bud-cone yellowish, becoming reddish below, \pm cylindric, 14-15 \times 3-4 mm., gradually long-attenuate, nearly glabrous, shining, sepals sparsely ascending-hirsute from white papillae and not densely spreading-glandular-pubescent. Sepal

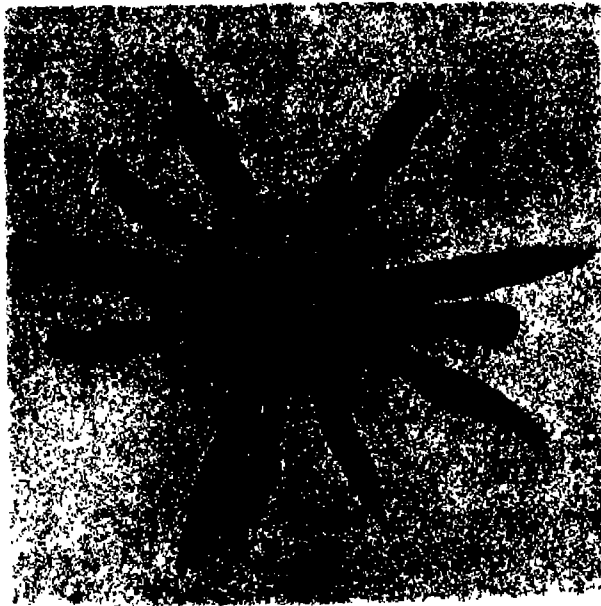


FIG. 63—*O. deflexa*, rosette, culture 65.33.

tips 1-2 mm., appressed, occasionally slightly divergent at apex, slightly hooded within, green tipped with red. Petals 10-12 \times 11-12 mm., widely overlapping, never opening out flat, petals bending outwards at top, truncate, toothed, and scarcely retuse. Filaments 8 mm., anthers 4 mm., usually touching only base of stigma in bud, stigma lobes 3-5 mm., spreading, base of stigma 6-9 mm. above hypanthium.

Diagnosis—Folia radicalia pallida viridia, anguste elliptico-oblongata, acuta, leviter bullata, fere undulata; costa alba rubicundotincta, rosula \pm evanida. Caulis altus, erectus, multis ramis caulinis instructus, rubrolineatus. Folia caulina arcuata, deflexa, acuta, rubro acumine, \pm concava, undulata, inferiora subpinnatifida ad basim. Ovarium viridopapillatum; hypanthium gracile; alabastra subcylindrica, attenuata, paene glabra, nitida. Apices sepalorum 1-2 mm. longi, appressi. Petala 10-12 mm. longa, truncata, multum superjacentia.

This very small-flowered species is marked by many distinctions, including (1) the relatively evanescent rosettes and bushy habit, (2) the foliage, (3) the rounded, subglabrous buds, very short sepal tips, and very small flowers. It does not appear to be nearly related to any other *Oenothera* described, but it probably occupies a large area in the Niagara peninsula of Ontario. The six original cultures, each from seeds of a different plant, showed an exceptional range of variation, which will now be described.

The original plant of culture 65.33 was collected by the roadside between Windsor



FIG. 64—*O. deflexa*, in flower, culture, 65.33.

and Amherstburg, Ont. It was noted as having small flowers, petals notched, sepals nearly glabrous, sepal tips very short, leaves narrow. The resulting cultures showed the following constant differences from the form here described as the type : (1) smaller, narrower stem leaves (13 cm. \times 25 mm. as against 23 cm. \times 39 mm.), plants less tall, (2) midribs pale pink, (3) flowers larger (petals 15–18 \times 18–19 mm.), hypanthium 32 \times 2 mm., ovary 12 \times 3.5 mm., petals not spreading outwards at top, stigma lobes at or even below the mouth of hypanthium tube. The fruits were long and slender, 37 \times 5 mm.

Cultures 67.33 and 68.33 were alike. They showed marked differences from the type and may be characterized as var. *bracteata* n. var. Both were from plants on waste land of the Yawkey Estate at Sandwich, Ont. The former plant was observed to have extremely narrow, small, cuneate, notched petals, and narrow leaves turning reddish. Observations of these cultures and their descendants show the following constant peculiarities of var. *bracteata*: (1) Plants larger, with larger rosette leaves (fig. 65) and stem leaves (16–17.5 cm. \times 35–47 mm.) not subpinnatifid, pink midribs, (2) bracts very foliaceous (14.5 cm. \times 38 mm.), falcate-arcuate-deflexed, overshadowing the buds and flowers, not curled or appreciably crinkled (fig. 66), (3) petals very narrow but not cruciate (*i.e.*, not linear), ranging from 13 \times 11 mm. to 8 \times 4 mm., bud-cone yellowish to pale red, rounded, 9 \times 5 mm., not tapering,

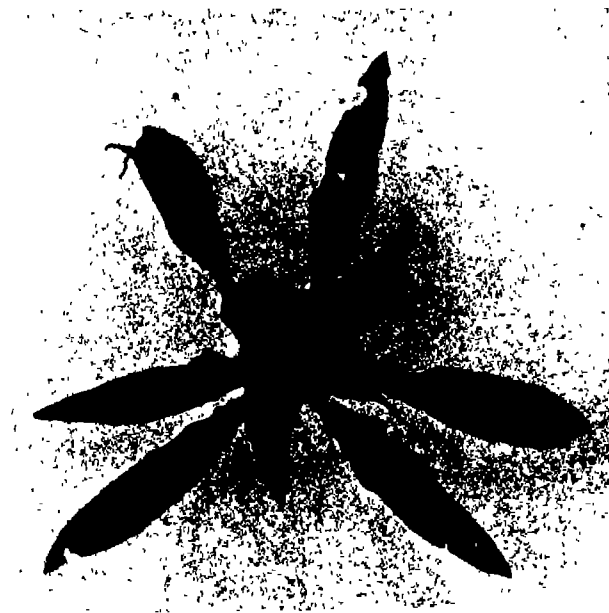


FIG. 65—*O. deflexa* var. *bracteata*, rosette, culture 68.33.

sepal tips 1–3 mm., terminal, subulate, spreading; stigma lobes long, 7–10 mm., spreading beyond petals.

Diagnosis—A specie sic differt: planta grandiora, folia ampliora, non subpinnatifida; bractae multum foliaceae, falcatae, arcuatae, deflexae; petala admodum angusta (11–13 mm. longa, 4–8 mm. lata), apices sepalorum terminales, subulati, extensi.

Culture 69.33 was derived from a very small plant on the same estate which had thin hypanthia and narrow leaves. The resulting culture had round liver-coloured spots on the leaves, which were not found in other strains. Culture 71.33 and its descendants, derived from a tall plant growing among grass in the village at Colchester, Ont., agreed with 65.33 and its descendants except in having smaller, narrow

petals ($12-14 \times 8-9$ mm.). All the differences mentioned are constant for each culture and thus represent inherited differences. They are probably due in certain cases to single genes, but more often to sets of linked genes. The most marked variations in this species are in size of flower, width of petal, size of bracts, and presence or absence of red on the midribs. The variation in petal-width is of quite a different kind from that seen in cruciate flowers, the latter being, in some cases at least, a single gene difference. In this species the petals never become linear,



FIG. 66—*O. deflexa* var. *bracteata*, in flower, late type, culture 67.33.

but narrowly cuncate, and the difference from broad petal is perhaps not due to a single gene.

The only other marked variation in this series of cultures was a plant in culture 63.33 which was a chimera having white leaf-margins and blotches of whitish tissue.

This species, like several others, tends to put up a stem early under the conditions of cultivation, so that the rosette may be almost omitted. Frequently in a culture

some plants are of this "early" type while others remain much longer in the rosette stage and belong to the "late" type. The evidence indicates, however, that this difference is not inherited but that it is purely a physiological reaction to the environmental conditions at a certain stage of development. The seeds tend to germinate badly in cultivation and so two of the strains were lost.

O. insignis BARTL.

From seeds collected by Professor W. P. THOMPSON on the golf course at Saskatoon, Sask., Canada, 18 September, 1933. Sixteen plants were grown as culture 14.34. They were uniform except for one typical *lata* mutation (fig. 69), and a full description is given, as it supplements the one already published (BARTLETT, 1914*b*).



FIG. 67—*O. insignis* Bartl., from Saskatoon, rosette, culture 14.34.

Description—Rosette leaves pale grey-green, *ca.* 19–21, appressed to the soil, elliptic-ovate, apex apiculate from a rounded or obtuse apex, or merely acute, lamina 10–14 cm. \times 40–53 mm. (petiole 1.5–3 cm. long), nearly flat, crinkling obscure, \pm slightly undulate below, margin repand-dentate or subpinnatifid below, repand-denticulate above, midribs white, finely appressed-pubescent on both surfaces (fig. 67).

Stem erect or slightly bent at tip, in annual plants reaching only 40 cm. and producing flowers and fruits from the base, and even in the axils of the later rosette leaves. This is probably its natural habit on the Canadian prairies. In Regent's Park only two plants flowered the first year. Eleven of the remainder survived the winter and in 1935 produced tall, erect stems *ca.* 120–130 cm. high, rather strongly

ribbed in the upper part and often fasciated.* Numerous basal branches, shortly or far decumbent and arcuate-ascending. Cauline branches numerous, arcuate-ascending. Stem very pale green, occasionally tinged with mauve, inconspicuously arcuate-ascending or patulous-pilose from scattered reddish papillae and finely subappressed-crisped-puberulous.

Stem leaves deflexed, flattish, or slightly concave, dull greyish-bluish-green, slightly crinkled, lanceolate to narrowly elliptic-lanceolate, margin finely denticulate,



FIG. 68—*O. insignis* Bartl., from Saskatoon, in flower as biennial, culture, 14.34.

8–10 cm. \times 17–28 mm., midrib white, upper surface finely subappressed-puberulous with a few longer suberect hairs, lower surface similar but all hairs more suberect.

* A number of cultures remained rosettes in 1934 and produced fasciated stems in 1935 (fig. 68). *O. purpurea* KLEB., which can only be grown with us as a biennial, also always produces strongly fasciated stems. Fasciation in all these cases appears to be the result of the large persistent rosettes storing an excess of reserve material in the fleshy roots, this excess nourishment causing fasciation when the plant finally forms a stem in its second season.

Inflorescence not dense except towards apex. Lower bracts patulous or deflexed, concave, lanceolate, ovate-lanceolate or rarely ovate, rounded at base, 4-7 cm. \times 10-20 mm. Upper bracts spreading, ca. 15-30 \times 6-12 mm. Apex of inflorescence flattish or slightly depressed, comose, easily overtopped by highest developed buds.

Ovary 9-10 \times ca. 3 mm., densely ascending or \pm subappressed softly hirsute from brownish-red or green papillae. Shortly spreading glandular-pubescent, but this pubescence is mostly hidden. Hypanthium 21-24 \times 2 mm., rather sparsely ascending- or patulous-hirsute from green papillae and rather sparsely spreading glandular-pubescent. Bud-cone 11-12 \times 4.5-5.5 mm., yellowish-green, \pm obscurely quadrangular, attenuate into sepal tips with reddish lines descending from sinuses between the tips. Indumentum as on ovary, but hairs longer, more spreading, papillae green. Sepal tips terminal but hooded within, appressed or divergent at apex, 2-3 mm. long, sometimes red at apex. Petals ca. 10 \times 8-9 mm. (15-17 \times 15-20 mm. in annual plants), overlapping, truncate, flower cup-like, not opening widely, petals withering orange on margin.

Filaments 6-7 mm., anthers 6 mm., stigma lobes 3-4 mm., reaching nearly to top of anthers, appressed or spreading in anthesis. This strain from Saskatoon, Sask., nearly agrees with *O. insignis*, described by BARTLETT (1914b) from the shore of Lake Superior near Duluth, Minnesota.

No other species is known with the habit of flowering from the lowest stem nodes and the leaf-axils of the rosette. The differences between the two strains can only be fully determined by growing them side by side, but the following minor differences from the species appear to exist: (1) rosette leaves elliptic-obovate (shape not described in *O. insignis*), (2) bracts narrower, not petiolate, (3) sepal tips shorter, appressed, or divergent. This record indicates that the species is essentially a prairie form, extending from Saskatchewan to Lake Superior. It is evidently on the way to becoming acaulescent in habit, like many prairie species, as BARTLETT suggests, but when grown under English climatic conditions this sub-acaulous habit is nearly suppressed.

The *lata* mutation (fig. 69) occurring in wild seeds is of particular interest. It, and other cases recorded in this paper, shows that, whether they survive or not, trisomic mutations must occur not infrequently in nature. This rosette was an exact parallel to *O. Lamarckiana* mut. *lata* in leaf-shape, but in other respects the leaves agreed with those of the species. This plant survived the winter, but was killed by the heavy frosts in the spring of 1935. Since all the other rosettes of the culture survived and flowered, it is evident that the *lata* mutant was less resistant than the type of the species.

O. albinervis, n. sp.

From seeds collected in the town of Fargo, N. Dakota, at Kindred, N.D., and on open ground near sand dunes at Barrie, Richland Co., N. Dakota, 40 miles from Fargo, 15 October, 1932. When growing under these somewhat arid sandy conditions the leaves were narrow, smooth, and covered with a conspicuous silky pubescence.

In the climate and soil of Regent's Park, the silky pubescence failed to appear and the leaves become strongly crinkled. In this species, as in several others, "early" and "late" types are strongly marked. The early plants, which omit the rosette stage, were in culture 72.33 taller, lighter green, with smooth leaves and smaller, curled bracts; while the late plants produced a rosette followed by a stem, they were darker green, the stem leaves \pm crinkled, the bracts larger and less curled. The evidence from successive years of culture indicates that the early and late types, which have been observed in several *Oenothera* species and sometimes show striking differences, are not inherited but are simply an epharmonic response, occurring as the result of some reaction to environment early in the development of the

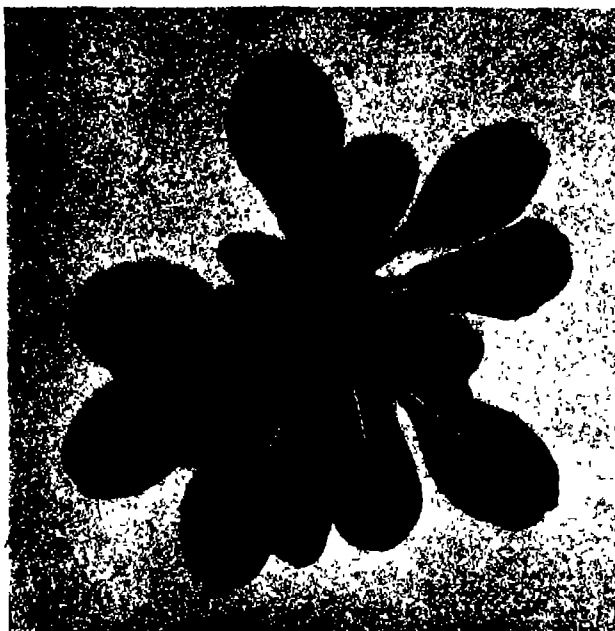


FIG. 69—*O. insignis* mut. *lata*, rosette, culture 14.34.

young plant. Culture 75 and its descendants from Barrie, N.D., will be treated as the type culture.

TABLE XXVI

	Fargo	Kindred	Barrie
1933	72 (50)	73 (50)	75 (50)
1934	96 (2)	97 (6)	98 (4)
1935	99 (40)		100 (10)
			101 (17)

Description—Rosette leaves reaching 23 cm. \times 70 mm., elliptic-oblongate, apex acute, or obtuse with acute point, usually strongly crinkled and margin strongly undulate, obscurely and distantly repand-denticulate, glands green, scarcely

visible, markedly repand-dentate towards the base, usually with large reddish patches, very sparsely suberect-pubescent above, pubescence longer below, midrib white (fig. 70).

Stem erect, *ca.* 90-100 cm., strongly ribbed, green, sparsely \pm appressed or patulous-hirsute with usually only green papillae or none, densely suberect or arcuate-subappressed-pubescent, basal branches many or none, appressed prostrate and arcuate, the apices ascending, often nearly as long as central stem, pinkish



FIG. 70—*O. albinervis*, rosette, culture 100.34.

towards base, cauline branches usually none. Bark brown at base of stem, excorticating. Stem leaves 11.5-21 cm. \times 35-60 mm., deflexed, upper often very steeply, lanceolate or elliptic-lanceolate, strongly crinkled, lower usually with large reddish patches, margin wavy, repand-dentate in lower two-thirds (lower sub-pinnatifid at base) repand-denticulate above, glands green or reddish, both surfaces densely suberect-pubescent, midrib white, \pm subappressed-cripsed-pubescent above, sparsely pilose and densely \pm subappressed pubescent below.

Lower bracts lanceolate, arcuate-deflexed, very concave, wavy, and crinkled, 5-6.5 \times 2-2.5 cm. Upper bracts arcuate-spreading, curled, up to *ca.* 1 cm. long and

1 cm. wide. Apex of inflorescence flattish or convex, comose, overtopped by highest developed bud-cones. Spike fairly dense. Ovary $12-15 \times 3.5$ mm., densely hoary and subappressed pubescent (no red papillae), hypanthium $20-37 \times 2.5-3$ mm., stout, patulous-ascending hirsute, arcuate-subappressed-pubescent and spreading glandular-pubescent, remaining stiff when petals wilt. Bud-cone green, \pm cylindric, $11-15 \times 5-7$ mm., hoary and densely subappressed pilose-pubescent,

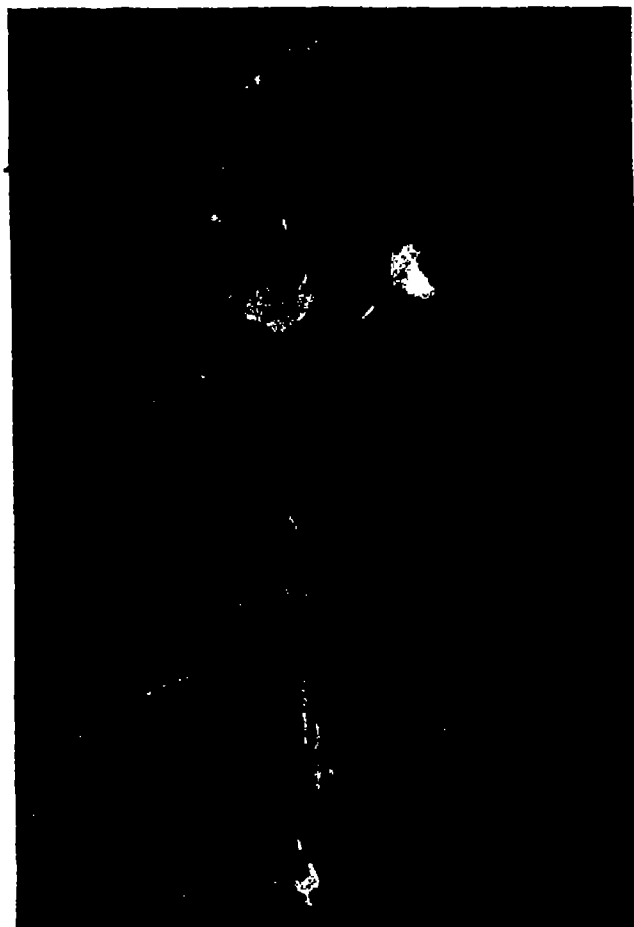


FIG. 71—*O. albinervis*, in flower, culture 100.34.

sepal tips 3-4 mm., terminal, appressed or divergent, with a touch of red at the extreme tip. Petals $13-18 \times 14-23$ mm., truncate and irregularly toothed, opening to cup-shape, overlapping. Base of stigma 0.2 mm. above hypanthium, stigma lobes 5-9 mm. long, appressed or spreading, filaments 9-11 mm., anthers 6-7 mm., slightly overtopping stigma lobes (fig. 71).

Fruits green, long, 40×7 mm., with variable numbers of long and short hairs.

Diagnosis—Folia radicalia elliptico-lanceolata, plerumque valde bullata, margine admodum undulata, obscure repando-denticulata, glandulis viridibus, vix evidentibus,

repando-dentata ad basim, fere cum magnis maculis rubescentibus, costa alba. Caulis erectus, viridis, papillae fere virides aut non. Folia caulina valde bullata, similia foliis radicalibus. Ovarium 12-15 mm. longum non rubropapillatum, hypanthium robustum, cum pilis patulis ascendentibus. Alabastra subcylindrica, viridis, apices sepalorum 3-4 mm. longi, terminales, rubrotincta in extremo acumine. Petala 13-18 mm. longa, superjacentia, stigmatis basis paulum suprahypanthium.

The foliage, particularly of the cultures from Kindred, was exactly like that of *O. mut. rubrinervis* DE VRIES, except that the midribs were white. This species differs very markedly, however, having white midribs, small flowers, and differences in stem apex and in habit. It is markedly unlike *O. strigosa* (RYD.) MACK. and BUSH, which occurs in this region, and even more sharply separated from the new Dakotan species, *O. rubricapitata*. *O. albinervis* is evidently widely distributed in North Dakota.

The 1933 cultures of this species showed marked "early" and "late" types, which could be scored without difficulty in cultures 72 and 73, but in culture 75 the differences were not marked enough for scoring. The early type is taller with lighter green foliage, leaves not crinkled, bracts smaller, curled. The late type has darker green foliage, leaves \pm crinkled, bracts larger, less curled. Cultures 96 and 97 of 1934 were derived from selfing late and early plants respectively of culture 72.33. The resulting offspring were all alike, producing good rosettes, some of which did not flower until 1935. Hence the "early" condition is not inherited.

In the 1935 cultures certain interesting differences were observed between 99 and 101, which had no doubt been overlooked in the previous years owing to less minute observation. (1) In 99 the petals turned dirty orange colour in fading, while in 101 they did not change colour in fading, but every petal in every plant had a small pale orange spot at the base on the outside. This was first observed on 3 August, but the flowers opening on 8-14 August showed no trace of this spot. It is therefore an evanescent character, appearing in the Barrie strain, but not in that from Fargo. As cultures 99 and 101 were grown in rows side by side and the difference was constant when it appeared, it cannot be accounted for environmentally. Culture 99 differed from 101 in (2) having stem leaves slightly wider (16 cm. \times 47 mm. as against 16 cm. \times 43 mm.) and with patches of paler green between the lateral veins; (3) having a longer style (this difference persisted for at least 12 days); (4) bud-cone less stout (15 \times 6 mm.); (5) sepal tips not appressed, but spreading towards their apex. All these differences were constant throughout the two cultures.

O. rubricapitata n. sp.

A very distinct species, from seeds collected in a wooded area by a pond at Kindred, N. Dakota, some 30 miles from Fargo, 15 October, 1932.

Cultures : 1933	74 (50)
1934	99 (11)
1935	100 (6)

Description—Rosette leaves dull, somewhat greyish-green, oblanceolate, or elliptic-oblanceolate, reaching 24 cm. \times 57 mm. (petiole 3–5.5 cm. long), apex acute, shortly cuspidate or obtuse and apiculate, crinkling conspicuous, undulation usually present towards base, margin repand-dentate to sub-pinnatifid below, repand-denticulate to subentire above, glands not visible, midrib conspicuously pink, densely suberect or erect pubescence on both surfaces (fig. 72).

Stem *ca.* 60 cm. high, erect, strongly ribbed, green with purple patches, patulous-hirsute and arcuate-subappressed-pubescent. Few papillae, green or red in coloured



FIG. 72—*O. rubricapitata*, rosette, culture 99.34.

patches, excorticating at base in early type which omits the rosette. Long basal branches prostrate-decumbent, numerous short cauline branches \pm arcuate-ascending, usually deep red. Stem leaves \pm arcuate-deflexed, lanceolate, elliptic-lanceolate below to ovate-lanceolate above, flattish or convex, smooth or \pm crinkled near (red) midrib, 6.5–19 cm. \times 22–43 mm., lowest dentate to subpinnatifid at base, pubescence suberect, midrib \pm appressed-crispulous above. Bracts arcuate-spreading or strongly deflexed, lanceolate, cordate, sessile, lowest 9 cm. \times 40 mm., flattish; tip wavy, uppermost bracts very small; apex of inflorescence convex, overtopping highest developed buds, spike rather lax. Ovary 12 \times 2.5 mm., patulous-hirsute, arcuate-subappressed-pubescent and very sparsely spreading

glandular-pubescent, papillae green or sometimes reddish. Hypanthia \pm arcuate, $25-28 \times 2$ mm., sparsely spreading or patulous-hirsute and glandular-pubescent, papillae green. Bud-cone greenish-yellow, *upper quarter conspicuously deep red*, subcylindric, *ca.* 11×5 mm., sparsely patulous-hirsute and sparsely spreading glandular-pubescent, small red papillae on red portion of bud. Sepal tips 3-5 mm., green in upper, red in lower half, or throughout, stout, terminal, appressed or very



FIG. 73—*O. rubricapitata*, in flower, culture 99.34.

slightly divergent. Petals 13×13.5 mm., opening at 45° , rather widely overlapping, truncate, and "irregularly toothed". Base of stigma just above mouth of hypanthium, stigma lobes 3-4 mm. long, not separating, filaments *ca.* 9 mm., anthers 6-7 mm., far overtopping stigma lobes (fig. 73).

Diagnosis—Folia radicalia surda, aliquantum canoviridia oblanceolata aut elliptico-oblanceolata, acuta, breviter cuspidata aut obtusa et apiculata, bullata,

margine repandodentata ad subpinnatifida ad basim, costa rubicunda. Caulis erectus, viridis cum maculis purpureis, paucae papillae virides, aut rubrae in partibus purpureis caulis, exuens corticem ad basim.

Folia caulina inferiora lanceolata, ad basim dentata ad subpinnatifida, superiora ovato-lanceolata ; \pm bullata prope costam rubicundam. Spica sublaxa, hypanthia arcuata, sepala viridescencia-flava, quadrans superior insigniter atroruber. Apices sepalorum 3-5 mm. longi, robusti, terminales, appressi ; petala 13 mm. longa, stigmatis basis paulum supra hypanthium.

This species is very distinct and uniform, the ornamental red shoulders to the buds being a new character in the genus. The plants showed "early" and "late" types under the weather conditions of 1933 only. The foliage is curiously like that of *O. deflexa*, especially var. *bracteata*, but there is probably no very close relationship to that species. A plant in culture 74.33 was examined by Mr. FORD and found to have a ring of 14 chromosomes.

O. Lamarckiana Ser.

The origin and history of this species has been much discussed and will only be touched upon here, especially as the matter has lost its prime importance now that similar mutation phenomena have been described in various species taken directly from the wild, and whose wild origin there is no chance to dispute. DE VRIES (1914) and DAVIS (1927) have discussed at length the origin of *O. Lamarckiana*, and have published photographs of several herbarium specimens in the Museum d'Histoire Naturelle in Paris which bear on this question. It seems certain that both *O. grandiflora* SOL. and *O. Lamarckiana* SER. are represented in these specimens. I am in agreement with DE VRIES that his plate XVII represents *O. Lamarckiana* SER., but I incline to the view that his plate XVIII belongs to *O. grandiflora* SOL. On a recent visit to the Jardin des Plantes in Paris, the specimen represented by Plate XIX of DE VRIES's paper and by Plate V of DAVIS's was re-examined with particular interest. It was evidently grown from seeds collected by MICHAUX somewhere in eastern North America late in the eighteenth century. DE VRIES regarded it as belonging to the type of *O. Lamarckiana*, while DAVIS thought it was a very different species. I have compared some of its measurements and other characters with those of recently-made herbarium specimens from the DE VRIES strain of *O. Lamarckiana*.

This MICHAUX specimen differs in certain respects from the *Lamarckiana* of DE VRIES : (1) the stem leaves have petioles, which may be of considerable length, (2) the leaves are somewhat narrower, (3) they may be somewhat less crinkled, although this character is very difficult to assess in dried specimens, (4) the flowers were somewhat larger. The petioles on the middle stem leaves of this specimen were 8-10 mm. in length, a peculiar character not known in any other *Onagra*. Three leaves from the middle part of the stem measured respectively 12.5 cm. \times 27 mm., 12 cm. \times 33 mm., and (a lower leaf) 15 cm. \times 27 mm. (including petiole 2 cm. long). By

comparison, mid-stem leaves from dried specimens of *O. Lamarckiana* from my cultures of 1935 measured 10-11 cm. \times 33-34 mm. but were practically sessile. The buds are very similar in form and pubescence in the two types, as DAVIS also admits, the largest bud cones on the MICHAUX specimen being 40×9 mm. with sepal tips *ca.* 6 mm. long, whereas in my specimens they were somewhat smaller, bud-cone 34×10 -11 mm. with sepal tips 5-7 mm. In pubescence and other characters they were alike.

The only differences between the two types are therefore in the presence or absence of leaf petioles, a small difference in leaf-width and in flower-size, and some possible difference in crinkling. These differences are varietal only, and both types clearly belong to the same species. The contention of DAVIS that MICHAUX's plant represents a very distinct species is inadmissible, although it would evidently rank as a variety of *O. Lamarckiana*, distinguished from the strain of DE VRIES by certain well-marked differences. It is highly probable that wherever this strain of MICHAUX grew wild, the strain represented by the *O. Lamarckiana* of DE VRIES would not be far distant geographically.

The present genetic survey has brought out the fact that seeds of *O. Lamarckiana* agreeing with the English garden strain, which differs slightly in colouring and crinkling from that of DE VRIES's cultures, have not infrequently been introduced into private gardens in Eastern Canada, from whence they have sometimes escaped more or less successfully. In company with Professor MARIE-VICTORIN in 1932, we found at Lotbinière, above Quebec on the south side of the St. Lawrence, a small group of plants of this species growing on the edge of a field by the roadside. They were probably a recent escape from a garden in the neighbourhood and they showed no definite signs of spreading. Very different was the case of a colony found in September, 1935, at Barss Corner, Lunenburg Co., Nova Scotia. On a farm I described, some hundred yards from the road, a colony of *O. Lamarckiana* which was growing and spreading on a hillside in a pasture or hayfield among grass. About one hundred plants were counted, many in flower and others rosettes. This locality is almost in the middle of the peninsula of Nova Scotia, protected from the coastal winds. The climate is exceptionally mild and these plants flourish and spread rapidly. Enquiry from the owners of the farm elicited the information that seeds of this species had been obtained from a seed firm of New York about ten years ago. They have "grown like weeds ever since" and have "gone all over the farm". The success of this species in establishing itself and spreading even through pasture richly stocked with vegetation, indicates either that the home of this large-flowered species was further north than is generally supposed, or that the species is by no means narrowly adapted to a southern climate. It was particularly surprising to see an *Oenothera* succeeding in competition with grasses in an undisturbed soil.

In Table XXVII is given the known distribution of the various species investigated. No doubt the distribution of many of these species will be extended with further knowledge.

TABLE XXVII—DISTRIBUTION OF THE VARIOUS SPECIES

Species	Localities
<i>O. paralamarckiana</i>	Woods Hole, Massachusetts.
<i>O. pycnocarpa</i> ATK. and BARTL.	Ithaca, N.Y.
var. <i>parviflora</i>	Hamilton and Georgetown, N.Y.
var. <i>cleistogama</i>	Clinton, N.Y.
<i>O. novae-scotiae</i> GATES	Middleton, Annapolis Co., N.S., and vicinity.
var. <i>serratifolia</i>	Kentville, King's Co., N.S.
var. <i>distantifolia</i>	Newport, Hants Co., N.S.
<i>O. comosa</i>	Wilmot, Annapolis Co., N.S.
<i>O. intermedia</i>	Bear River, Digby Co., N.S.
<i>O. flecticaulis</i>	Mouth of Lahave River, Lunenburg Co., N.S.
<i>O. Hazelas</i>	Lockeport, Shelburne Co., and Wentworth, Cumberland Co., N.S.
var. <i>parviflora</i>	Port Mouton, Queen's Co., Chester, Lunenburg Co., and Middleton, Annapolis Co., N.S.
<i>O. subterminalis</i>	Higgins Brook, Cumberland Co., and North River, Colchester Co., N.S.
<i>O. grandifolia</i>	Wentworth and Port Howe, Cumberland Co., and Waugh's River, Colchester Co., N.S., and Point de Bute, Westmoreland Co., N.B.
<i>O. Royfraseri</i>	Sackville, Westmoreland Co., N.B.
<i>O. sackvillensis</i>	Sackville, N.B.
var. <i>albiviridia</i>	Sackville, N.B.
<i>O. ammophiloides</i>	Guysborough Co., N.S.
var. <i>laurensis</i>	Port Elgin, Westmoreland Co., N.B.
<i>O. parva</i>	Near the St. Lawrence, from Bic, Rimouski Co., to L'Islet, L'Islet Co., Quebec.
<i>O. leucophylla</i>	St. Valier, Bellechasse Co., and Berthier-en-bas, Montmagny Co., Quebec.
<i>O. biformiflora</i>	St. Valier, Bellechasse Co., Charny, Levis Co., and St. Antoine les Fonds, Lotbinière Co., Quebec.
<i>O. laevigata</i> BARTL.	White Sulphur Springs, W. Virginia.
var. <i>similis</i>	St. Valier, Quebec.
var. <i>rubripunctata</i>	St. Valier, Quebec.
<i>O. Victorini</i> GATES and CATCH.	St. Hubert, near Montreal.
var. <i>parviflora</i>	St. Anne, Kamouraska Co., St. Antoine les Fonds, Lotbinière Co., and St. Hubert, Montreal, Quebec.
var. <i>intermedia</i>	St. Valier, Ste. Anne de Bellevue, Jacques Cartier Co. and Cap Tourmente, Montmorency Co., Quebec.
var. <i>undulata</i>	Near Toronto, Ontario.
<i>O. angustissima</i> GATES	Ithaca, N.Y.
var. <i>quebecensis</i>	Cap Tourmente, Montmorency Co., Quebec.
<i>O. niagarensis</i>	Niagara Gorge, N.Y.
<i>O. repanddentata</i>	Colchester, Essex Co., Ont.
<i>O. deflexa</i>	Windsor, Ont., and vicinity (Essex Co.).
var. <i>bracteata</i>	Sandwich, Ont. (Essex Co.).
<i>O. insignis</i> BARTL.	Duluth, Minn., and Saskatoon, Sask.
<i>O. albinervis</i>	Fargo, Kindred and Barrie, N. Dakota.
<i>O. rubricapitata</i>	Kindred, N.D.

CATENATION

Material was collected by Mr. C. E. FORD from many of the 1933 cultures to determine their catenation. His results are given in Table XXVIII, each determination being made from a single plant. A ring of 14 chromosomes was present in the pollen mother cells in every case except *O. rhombipetala*, which has seven free pairs. This extensive sampling makes it highly probable that all the *Oenotheras* in eastern North America have a ring of 14.

TABLE XXVIII—CHROMOSOME CATENATION IN VARIOUS NEW SPECIES AND VARIETIES

	Culture
<i>O. paralamarckiana</i>	1.33
<i>O. novae-scotiae</i> with large flowers	8.33
<i>O. comosa</i>	5.33
<i>O. Hazelae</i> var. <i>parviflora</i>	87.33
<i>O. grandifolia</i>	21.33
<i>O. subterminalis</i>	13.33
<i>O. ammophiloides</i> var. <i>laurensis</i>	20.33
<i>O. parva</i>	25.33
<i>O. lasvigata</i> var. <i>similis</i>	28.33
" " " 	29.33
" " " 	31.33
" between var. <i>similis</i> and var. <i>rubripunctata</i> . . .	39.33
" var. <i>rubripunctata</i>	30.33
<i>O. leucophylla</i>	33.33
" 	36.33
" 	42.33
<i>O. Victorini</i> var. <i>intermedia</i>	43.33
Undescribed species from St. Jerome, near Montreal . . .	53.33
<i>O. Victorini</i> var. <i>undulata</i>	63.33
<i>O. rubricapitata</i>	74.33
Species from Lockeport, N.S., resembling <i>O. biennis</i> . . .	81.33
<i>O. rhombipetala</i>	109.34

MUTATIONS

Various general points regarding these cultures have been referred to in the introduction. Here it will be necessary to discuss first the mutations which have occurred in these wild species. For convenience of reference they are compiled in Table XXIX.

It was formerly often suggested that the mutations of *O. Lamarckiana* were in some sense a result of its cultivation or hybridity. In so far as a ring of chromosomes is a sign of the heterozygous condition, the latter suggestion is true, but it is shown in the present paper that a number of wild species produce similar mutations, even directly from wild seeds. Table XXIX is a summary of the mutations—all probably

trisomic except the periclinal chimaera and one which is triploid—produced in quite small cultures of seven different species. It is already known that wild species such as *O. novae-scotiae* and *O. eriensis* have a high percentage of non-disjunctions (SHEFFIELD, 1927), and it is now clear that other wild species have a high

TABLE XXIX—MUTATIONS FROM WILD SPECIES OF *Oenothera*

Species	Generation	Name	Nature of mutation
<i>O. paralamarckiana</i> *	P ₁	dwarf	trisomic ?
	P ₂	several	trisomics
<i>O. flecticaulis</i>	P ₁	linearis	trisomic ?
<i>O. sackvillensis</i>	P ₂ and P ₃	dwarf	trisomic
<i>O. ammophiloides</i> var. <i>laurensis</i>	P ₁ , P ₂ , P ₃	linearis	trisomic
<i>O. parva</i>	P ₁	yellow leaf-margin	periclinal chimaera
<i>O. parva</i>	P ₁ , P ₂	hebetifolia	trisomic ?
<i>O. biflora</i>	P ₁	triploid	21 chromosomes
<i>O. insignis</i>	P ₁	lata	trisomic

* For later mutations from this species see GATES and NANDI (1935).

frequency of trisomic mutations. In *O. paralamarckiana* at least it is much higher than in *O. Lamarckiana*, and it appears probable that it is higher also in certain other species here described.

The *lata* from *O. insignis* is in leaf-form an exact counterpart of the *lata* from *O. Lamarckiana* or *O. biennis*, which indicates that one pair of chromosomes is probably common to the three species. If a specialized species from the Canadian prairies has at least one pair of chromosomes in common with two species which probably originated near the Atlantic seaboard, this is a striking testimony to the stability of the germplasm in the genus. It is possible, however, that a less simple explanation of these parallel trisomics may ultimately be required. The question can only be answered by further breeding experiments.

The blunt-leaved mutant *hebetifolia* from *O. parva*, on the other hand, has no known parallel, although it resembles *O. Lamarckiana* mut. *oblonga* in some respects. That *O. flecticaulis* and *O. ammophiloides* var. *laurensis* both produce linear-leaved trisomics is not surprising, since both are coastal forms related to *O. ammophiloides*. It is of interest that *O. paralamarckiana* and *O. sackvillensis*, which show no obvious relationship, should both produce trisomic dwarfs. These dwarfs differ in their foliage, however, and it is not necessary to conclude that they have the same chromosome in common. Perhaps they have in common only a dwarf gene, which becomes dominant when triplicated.

THE ST. VALIER COLONY

As will be seen from Table XXVII, two species of *Oenothera* frequently occur in the same locality, but St. Valier on the south shore of the St. Lawrence is remarkable as the meeting place of at least four very distinct species with four varieties. This

large colony extends for some distance along the freshwater estuarine shore of the St. Lawrence at the mouth of the River Boyer in gravel and shale, not sand. The forms found here have been classified as *O. leucophylla*, *O. laevigata* var. *similis* and var. *rubripunctata*, *O. biformiflora* and its variety *cruciata*, with *O. Victorini* var. *intermedia*. Of these forms, *O. leucophylla* has some resemblances to *O. eriensis* further west, but nearer relations with *O. ammophiloides* in the Gulf of St. Lawrence. *O. laevigata* originates from Virginia, *O. biformiflora* shows some connexions with Massachusetts and Vermont, while *O. Victorini* spreads westwards to Montreal, and beyond. In the small area at St. Valier all these forms are growing freely together, and there is definite evidence that the two varieties of *O. laevigata* at least occasionally intercross.

In 1933, 17 cultures were grown from St. Valier, each from seeds of a different wild plant, and in 1934 the same number of P_1 cultures was grown from selfed plants of the previous generation. The following year six further (P_2) cultures were studied, to confirm various characters observed. Of the 17 original cultures, many proved to be identical through two or three generations; but as it is impossible to be certain about the significance of differences observed in the field until they have been carefully studied in the experimental garden, it is quite possible that the wealth of forms in this colony is by no means exhausted by the present studies.

THE RATE OF EVOLUTION

Finally, it remains to discuss briefly the nature of the species and the principles of specific differentiation in the genus *Oenothera*. The principles of phylogeny in the genus were considered elsewhere (GATES, 1933) and are generally confirmed and extended by the present results, which indicate that lines of relationship can be traced north and south for *O. laevigata* BARTL. from West Virginia, and its varieties *similis* and *rubripunctata* from St. Valier, Quebec; *O. angustissima* GATES from Ithaca, New York, and its variety *quebecensis* from the north shore of the St. Lawrence; *O. biformiflora* from the St. Lawrence, whose nearest relatives appear to be in Vermont and Massachusetts. In Eastern Canada there appears to be a coastal series consisting of *O. flecticaulis*, *O. ammophiloides* and its var. *laurensis*, *O. parva* and *leucophylla*, all having strongly bent stems and many red, light-sensitive papillae on the sepals and ovaries. In September, 1935, about 60 more collections of seeds were made in Eastern Canada, which will fill in many lacunae. Further discussion of this aspect is postponed until they have been investigated.

The present study has been sufficiently intensive in certain areas to give a fuller picture than has hitherto been attained of the rich multiformity in the genus and particularly in certain local populations. In some localities there appears to be a single form showing only the most minute type of genetic variations, with in some cases one or two other genes for such conditions as red or white midribs. In other areas the population is much more multiform, reaching a maximum with several species and their variations represented. The cause of this multiformity must be

sought in gene mutations, but it must be remembered that an *Oenothera* population is very different from an ordinary open-pollinated Mendelian population. Among the small-flowered forms here described, crossing must be a relatively rare occurrence, and there is little or no segregation, each individual, although heterozygous for a large number of factors, breeding true owing to the chromosome catenation. Crossing therefore produces a new type generally more or less intermediate between the parents, or rather between the two uniting complexes. Further genetic analysis of all these species must therefore be undertaken to determine their complexes, and many crosses for this purpose have already been made.

The wealth of multiformity in these cultures and the number of characters new to the genus have been somewhat surprising. The diversity is much greater than in some other genera occupying the same area. This leads to the question of relative rates of evolution. It seems necessary to conclude that evolution or differentiation of types has taken place much more rapidly in *Oenothera* than in many other genera. FERNALD (1925), in his extensive studies of the flora of Eastern Canada, has shown that a series of areas around the Gulf of St. Lawrence, including the Long Range of Western Newfoundland, the Torngat Mountains of north-eastern Labrador, considerable parts (Shickshock Mountains) of the Gaspé Peninsula, the Magdalen Islands, much of Prince Edward Island, and the north-eastern end of Cape Breton Island, which escaped glaciation more or less completely, are the home of many endemic plant species which are only found elsewhere in such distant regions as the Rocky Mountains or Greenland. Fernald concludes that these plants persisted through the Ice Age on local areas and nunataks in Eastern Canada. He finds that these lands around the Gulf of St. Lawrence have 81 such endemic species, in strong contrast with the White Mountains further south which have only 3 endemic species, and Nova Scotia which was thoroughly glaciated and has but one endemic, *Agalinis neoscotica* (GREENE) FERNALD, whose specific status is somewhat doubtful. It is generally recognized that the Atlantic slope of Nova Scotia derived its flora *via* the submerged Atlantic continental shelf from the southern Coastal Plain. FERNALD states that its species are "nearly all typical of the Coastal Plain region from New Jersey to Florida and Mississippi in a much warmer climate". Besides the above-mentioned species, which may be only a variety of *A. purpercula*, the greatest departures from the southern types are "divergencies in pubescence, size, texture or habit, but not in fundamental reproductive characters". Eleven such geographic varieties have been recognized as endemic derivatives from the southern Coastal Plain. He concludes that "the best that nature has been able to do in 25,000 years with species of Alabama, Florida, and the Carolinas in the bleak climate of Atlantic Nova Scotia is to lengthen the trichomes or slightly to modify the foliage".

Regarding these views, it may be remarked that 25,000 years appears to be rather an excessive time allowance, and that other botanists are not agreed that "fundamental reproductive characters" are alone worthy of recognition as specific characters. Nevertheless, FERNALD's views appear fairly moderate and representative

of taxonomic opinion regarding specific differences among Angiosperms in general. Now I have described eight species and three varieties from the parts of Nova Scotia already explored. Clearly these species are either founded on smaller differences in general than those which systematists customarily recognize, or else evolution has proceeded at a more rapid pace in *Oenothera* than in most other genera. As regards the first alternative, while I have by no means taken the attitude of the extreme "splitter", yet some of the species here described are probably based upon smaller differences than the herbarium taxonomist would recognize. On the other hand, it is impossible to believe that any taxonomist would refuse specific rank to forms as distinct as *O. novae-scotiae*, *O. grandifolia*, *O. Hazelae*, and *O. ammophiloides*, all from Nova Scotia. It therefore seems necessary to conclude that evolution or differentiation of species has proceeded in the genus *Oenothera* at an exceptionally rapid pace. Before a final opinion can be formed on this matter, however, a corresponding genetic survey should be made of some relatively stable genus, collecting forms from many localities and studying their geographic variation in cultures.

SUMMARY

The present work is the beginning of a genetic survey of the genus *Oenothera* mainly in Eastern Canada and adjacent territory. It combines genetical with taxonomic methods, describing 17 new species, 15 new varieties, and many smaller variations, as well as recognizing 7 already described species in new localities, as a result of three years of pedigree cultures from about 100 collections of wild seeds from different localities.

Much light is thrown upon the geographic distribution and relationships of the various forms, indicating south to north movements in several different lines, and also a coastal series of forms. Six species have produced known or probable trisomic mutations, one species a triploid mutation and one a periclinal chimera (Table XXIX), most of these mutations being directly from wild seeds.

Plants of "early" and "late" development occur in cultures of several species, but the condition is not inherited and appears to be an epharmonic response to the environmental conditions occurring at an early stage of the young plant's development. The prairie species, *O. insignis* and *albinervis*, show marked alterations when grown in the English climate, the former losing its sub-acauliscent habit and developing long internodes, while the latter loses its silky pubescence and its leaves become crinkled.

In several species a new category of *evanescent* characters appears, such as the development of a pale red spot at the base of all the petals during a part only of the flowering season in one strain of *O. albinervis*; a touch of red at the base of the stem leaves in *O. niagarensis* during part of the season; and orange coloured filaments in the flowers at the end of the season only, in an undescribed species from near Montreal.

From numerous samples (Table XXVIII), the catenation of all these forms is found by Mr. C. E. Ford to be, without exception, a ring of 14 chromosomes.

Several large colonies of *Oenothera* have been studied by these methods, especially one at St. Valier on the St. Lawrence, where at least four species with four varieties were found. The relationships of these species were with the east, the west, and the south.

The polymorphic character of the genus, even in areas such as Nova Scotia, which were heavily glaciated and are believed to contain no other endemic species but only varieties, leads to the conclusion that evolution has proceeded more rapidly in this genus than in most others. Gene mutations have been active in supplying the raw materials for specific differentiation. Crossing has also probably played a part in increasing the number of specific types, since the hybrids breed true owing to catenation. Such new constant types are indistinguishable from the older species. Parallel mutations have also occurred many times in the different species. Their great importance in evolution has not yet been fully recognized.

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IX—The Influence of Moonlight on the Activity of Certain Nocturnal Insects, Particularly of the Family Noctuidae, as Indicated by a Light Trap

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(Communicated by Sir JOHN RUSSELL, F.R.S.—Received 24 January, Revised 16 March, Read 21 May, 1936)

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INTRODUCTION

For many years amateur entomologists have considered that on nights of full moon it is of little use going out to catch specimens, as insects will be few in number. This belief applies to all methods of collecting, including bait (sugaring) and light, and is supposed to apply particularly to the Lepidoptera.

Scattered through the literature on Agricultural Entomology one finds occasionally references to the use of light traps for the destruction of pests, and statements, usually from the tropics, that the catches were less at times of full moon; but so far as I am aware no proper statistical study of the question has ever been made.

One of the most striking series of figures is that produced by PAGDEN (1932) by trapping with a light trap the two Pyralid moths *Diatraea auricilia* and *Schoenobius incertellus* which are pests of rice in Malaya. He found, between 18 January and 29 June, 1931, six periods of maximum catch in both sexes of both species corresponding more or less to the no moon periods, and six periods of minimum catch corresponding even more definitely to the full moons. Scarcely any insects were captured at the time of full moon.

It has been known for many years that some animals have periodicities of activity corresponding to lunar months. Information about these has been dealt with in recent years chiefly by Fox (1923). The animals concerned are mostly marine, and it is probable that in some cases the effect is produced through the tides; but DORR (1932) has suggested that there is a lunar influence on the dates of movements of migrant birds. Specific reference to lunar influence on insects, other than captures by means of traps, are rare. HORA (1927) has suggested that some species of May-Flies (Ephemeridae) tend to emerge and make their nuptial flights at definite phases of the moon. The evidence that he brings forward is, however, too slight to be in any way conclusive, and a series of regular observations should be carried out in some suitable locality to see if the theory is supported.

THE ROTHAMSTED EXPERIMENTS

The Light Trap

In March, 1933, a light trap was started in one of the fields at Rothamsted Experimental Station, about twenty-five miles north of London, and it has been in practically continuous use for nearly three years. The trap was similar to that first designed in Egypt (WILLIAMS, 1924) except that an electric light of about 300 candle-power was used instead of acetylene, and an arrangement was added which divided the catch into eight equal periods during the night, so that it is possible to estimate the time of entry of the insects into the trap and hence the time of activity of any group or species. A full description will be found in a more recent paper (WILLIAMS, 1935).

The main object of the experiment was to provide material for a statistical examination of the relation of insect activity to climatic and weather conditions, but it is possible to use the figures so obtained to see if there is evidence of lunar periodicity in any or all of the groups into which the catch was divided.

The Measurement of Night Cloud and Moonlight

At an early stage in the investigation it was realized that the "moon" issue was associated with and complicated by, the presence or absence of cloud in the sky in two ways. First, the belief by amateurs that catches were low on moonlight nights might be an example of false reasoning, as nights on which the moon is obvious must be largely clear nights, and these are colder than cloudy nights owing to high radiation. It might therefore easily be that the lower temperature and not the moonlight was causing the low catch.

Secondly, if it is the moonlight that is affecting the catches, then its influence would not be expected to be so great on cloudy nights as on clear ones.

It was necessary, therefore, to have some measure of the cloudiness of each night. This was eventually done in five different ways.

(1) For the first year a note was kept each morning of all that could be recollected of cloud conditions in the late evening and early morning by members of the staff. This was at the best very unsatisfactory.

(2) Towards the end of the first year a "night cloud recorder" was built on the principle of the instrument in use at Greenwich (ANON, 1931). This is a long focus camera which photographs the pole star during the night, and from the tracing so produced the duration of the cloud obscuring the star can be measured. The modifications that were introduced at Rothamsted were chiefly in using a very much smaller camera with a roll film, and in having an automatic clockwork shutter, so that no attention was needed during the night. A description of the modified instrument is being published elsewhere (WILLIAMS, 1936, a).

(3) In order to supply more reliable information about the cloudiness of the nights in the first year, Greenwich Observatory, which is about 27 miles away to the S.S.E., kindly gave us a list of the nights in both 1933 and 1934, when their instrument indicated either less than 10% of the night cloudy (referred to below as "clear") or more than 90% of the night cloudy (referred to below as "cloudy" nights). A comparison of the results in the second year with our Rothamsted figures showed a close resemblance so that, in the absence of records at Rothamsted, the Greenwich figures could be used with a fair degree of accuracy.

(4) It was found that there was a certain correlation between the amount of night cloud and the difference between the "air minimum" and the "grass minimum" temperature for that night. This correlation is by no means absolute, but on the whole clear nights tended to have a high difference and cloudy nights a low difference. In spite, however, of many exceptions to the above general rule it was found that in the year 1934, if the difference between the air and grass-minimum

was 2° F. or less, then the night was always cloudy. This relation was therefore used retrospectively for 1933.

(5) The light of the moon was measured directly during most of 1934 and 1935 by a photographic instrument specially designed for the purpose. The instrument has been recently fully described (WILLIAMS and EMERY, 1935) and it is unnecessary to go into detail here. The principle is that a line image of the moon, produced by a cylindrical lens, is focussed on to a fixed strip of photographic paper. As the moon moves through the sky the lens follows its direction by means of a clockwork mechanism and the light from the moon thus forms a band on the sensitive strip. When the moonlight is bright the strip is darkened and when there is no moon the strip is unaffected.

A comparison of the results of the star recorder and the moonlight recorder shows that the former is much more sensitive to cloud. The clouds that obscure the pole-star still allow a considerable amount of light to pass from the moon. In fact, measurable light from the moon penetrates all but the thickest cloud. This is supported by the results below, which show that the lunar influence on certain insects is detectable even on cloudy nights.

To summarize—the five methods used for obtaining a measure of the night cloud were (1) personal observation, (2) Rothamsted star camera (1934 and 1935), (3) Greenwich star camera, (4) difference between air and grass minimum temperature, and (5) photographic moonlight recorder (1934 and 1935).

Note on the Apparent Movements of the Moon

The moon changes from its highest position in the sky at southing to its lowest and back again during a lunar month. In the summer it is low at full moon and high at no moon, while in the winter it is high at full moon and low at no moon. This results in the light of the full moon being much brighter in winter than in summer. The greatest angular height of the moon at southing above the horizon at London (latitude approx. 51° N.) is 66° and the lowest is 12°.

The moon souths on an average 49 minutes later each night, but within the course of a single lunar month the difference in time may vary from 41 to 66 minutes in a sequence which has two maxima and two minima.

The time of rising or of setting of the moon is later on an average each successive day by the same period (49 minutes) as that of the moon southing, but the variation is much greater and the extremes are approximately from 12 minutes to 1 hour 33 minutes. In each lunar month there is a cycle changing from long differences to short differences and then back again. In December the short differences occur when the moon is rising or setting about midday and the long differences when it is rising or setting during the night. In June the reverse occurs (*see* fig. 3). When the short differences occur during the night on one side of midnight with longer differences on the other side, there is an asymmetry of the lunar influence on successive nights which is discussed more fully later.

Any lunar effect on the activity of insects during the night would be expected to depend on : (A) the duration of the moonlight ; and (B) the intensity of the moonlight. The latter will depend on (1) the phase of the moon and (2) the angle of the moon above the horizon, and the latter again will depend on (a) the maximum height of the moon above the horizon (at southing) for the night and (b) the particular hour between southing and moon-rise or moon-set.

Note on the Use of Logarithms in Analysis

In the statistical treatment of a number of values for captures of insects such as that which follows, it is frequently necessary to combine together the captures on a series of days which have some condition in common, and to compare the total or average with that of a second series of days with different conditions.

The catches, however, vary very considerably from day to day, and if the actual numbers are added together and the arithmetical mean used, there is danger of the results being swamped by one or two abnormally high catches. For example, in the full moon week of October, 1933, the captures of Noctuidae on the seven nights were 0 : 0 : 1 : 62 : 0 : 0 : 0 ; while in the corresponding no moon week the captures were 2 : 4 : 0 : 0 : 10 : 3 : 3. The higher total for the full moon week is obviously unduly weighted by the single large catch.

Further, when the departures of series of values for catches from the arithmetical mean are studied, it is found that they consist of a large number of small negative departures and a few large positive departures. This gives a skew distribution which does not lend itself to statistical investigation by the normal formulae of standard deviation, etc.

If the logarithm of each value is taken and these summed and averaged, a measure is obtained of the geometric mean of the values. When this is done it is found that the swamping effect of the single large values is much reduced ; and if the log values are expressed as departures from a mean, the distribution of departures gives a much more normal curve.

This shows that changes in numbers are equivalent at different levels if they are in similar geometric and not arithmetic proportion. For example, that the change from 100 to 150 insects is equivalent to that from 1000 to 1500 and not to the change from 1000 to 1050.

This result was to be expected from *a priori* reasoning, as it is most probable that variations in climatic conditions would produce similar proportional changes in different populations, but it was necessary to confirm it by actual results before adopting the method of logarithms for general analysis.

A complication ensues if any of the values to be dealt with are zero, as the log of zero is minus infinity and the geometric mean of any series involving zero is itself zero. To overcome this it has been found possible to add one unit to all the values before converting into logs, and then to subtract the unit later when the log is reconverted to an anti-log.

The use of logarithms in the present series of figures emphasizes all differences that are consistent and reduces all that are not so. Thus in the two weeks' figures for the Noctuidae given above the sums of the numbers are 63 : 22, but sum of $\log (n + 1)$ are 2.10 : 3.89.

The method has been used in the interpretation of figures showing the time of flight of insects at night (WILLIAMS, 1935), and always gives more consistent results than does the use of the actual numbers themselves. This point is dealt with more fully in a separate publication (Williams, 1936, b.)

In the present study, therefore, the figures used for comparisons are (unless otherwise stated) $\log (n + 1)$ for a single value or $\Sigma \log (n + 1)$ for the value of a series.

When the series to be examined is spread over a considerable length of time, during which the total population is likely to have changed, a running mean is made of the $\log (n + 1)$ and each night expressed as a departure from that mean. The running mean here used is a 29-day mean, equal to the length of the lunar period, so as to eliminate variations of a longer period than the moon, but to leave the lunar period unaffected.

ANALYSIS OF THE RESULTS IN THE FAMILY NOCTUIDAE (LEPIDOPTERA)

In the past, most supposed effects of moonlight on insects have been reported in the Lepidoptera, so that the first investigation was made on this group. In order, however, that any results should be reliable statistically, it was necessary to obtain figures that would cover a number of lunar periods. No single species of Lepidoptera which was common in the trap complied with this demand, most lasting for only one or two lunar periods or less. It was therefore decided to study first the possible effect on the total numbers of all species of the family Noctuidae (s.l.).

If all species so combined are affected similarly by the moon then the effect should be noticeable in the combined results. If some species are affected and others not, the combined results would then show a diluted effect. The only difficulty in interpretation would arise if no effect was found in the group; as this might mean that there was no effect on any species or alternatively that some were affected positively and an equal number negatively, the two cancelling out each other in the combined total. This, however, did not occur.

There is no doubt that the most interesting results will be finally obtained by investigating single species or even each sex of a species separately, but for this purpose either many years' consecutive work will be necessary with traps in different localities or else some long- or many-brooded species must be chosen, probably in the tropics, such as the Rice borers investigated in Malaya by PAGDEN.

Numbers, Species, and Time of Flight of Noctuidae

In the course of the three years (1933, 1934, and 1935), 8712 Noctuidae were captured in the trap during the six summer lunar months discussed. The numbers in each year were 1582 in 1933, 1869 in 1934, and 5261 in 1935.

About 110 species were represented in the captures, and Table I shows the 25 species of which more than 50 specimens were captured, together with the total numbers of these in the three years. All these species occurred in each of the three years. The nomenclature is, for convenience, that used in South's "Moths of the British Isles".

These species account for 7838 individuals out of the 8712 captured, leaving 874 individuals distributed over about 85 other species.

TABLE I

More Abundant Species and Numbers of Noctuidae Captured in the Trap in the Three Years 1933-1935

<i>Agrotis exclamationis</i>	2074	<i>Miana fasciuncula</i>	148
<i>Amathes lychnidis</i>	781	<i>Cerigo matura</i>	146
<i>Xylophasia monoglypha</i>	723	<i>Taeniocampa gothica</i>	137
<i>Luperina testacea</i>	493	<i>Rusina tenebrosa</i>	124
<i>Noctua xanthographa</i>	483	<i>Leucania conigera</i>	112
<i>Noctua c-nigrum</i>	469	<i>Miana strigilis</i>	102
<i>Anchocelis lunasa</i>	419	<i>Leucania comma</i>	85
<i>Apamea secalis</i>	289	<i>Noctua primulas</i>	83
<i>Epineuronia popularis</i>	275	<i>Grammesia trigrammica</i>	73
<i>Noctua rubi</i>	227	<i>Agrotis puta</i>	55
<i>Leucania pallens</i>	206	<i>Hydroecia micacea</i>	51
<i>Leucania impura</i>	184	<i>Triphaena pronuba</i>	50
<i>Miana bicoloria</i>	164		

The Noctuidae in general are rather late flyers. In the three years, the distribution in the eight equal periods of the night, four before and four after midnight, was as follows: 599 : 826 : 1013 : 1454 : 1759 : 1429 : 955 : 309.

This gives a total of 3892 (47%) before midnight and 4452 (53%) after midnight with the maximum flight just after midnight. These figures are shown graphically in the vertical columns in fig. 5 together with similar ones prepared by a summation of the logarithms.

Table II shows the Noctuidae captured each night in six lunar periods (from full moon to full moon) in 1932 and summaries of the captures in 1934 and 1935, approximately from the beginning of May to the end of October in each year, when the Noctuidae were most numerous. This gives the possibility of investigating eighteen lunar periods.

Comparison of Full Moon and No Moon Weeks

Since if any lunar effect was present it would be expected to be most obvious near the two extremes of full and no moon, a simple comparison can first be made by adding in each lunar month the captures for the first four and last three days (the "full moon" week), and comparing them with the figures for the middle seven days (the "no moon" week). Table III shows the results for the eighteen lunar months, the larger figure of each pair being in heavy type. The table shows first

TABLE II

Noctuidae Captured in Each Day of Six Lunar Months of the Summer of 1933, with Summaries of the Captures for 1934 and 1935

Lunar Month Starting	9 May	7 June	6 July	5 Aug.	3 Sept.	3 Oct.	1933 Total	1934 Total	1935 Total	1933-34-35	
										Total	Five-day Smoothed mean
(Full	1	0	9	7	0	0	24	20	23	67	3.7
	2	0	7	8	4	0	28	12	71	111	3.8
	3	1	6	—	9	1	24*	14	54	92	4.4
	4	2	12	3	4	62	91	29	60	180	5.0
	5	2	9	—	4	25	54*	52	97	203	5.7
	6	1	5	5	3	2	31	51	78	160	6.6
	7	4	30	4	3	2	60	60*	106	226	7.5
	8	2	21	12	1	2	38	45	137	220	8.6
	9	1	39	1	9	1	63	48	212	323	11.2
	10	6	6	11	6	0	53	68	234	355	11.9
	11	3	4	5	4	5	36	82	445	563	12.4
	12	2	5	14	4	2	36	78	216	330	12.5
	13	2	5	6	8	4	31	73	182	286	12.5
	14	8	7	17	15	0	62	92	186	340	12.9
	15	3	8	17	7	0	42	92	216	350	13.3
	16	2	8	8	9	10	53	74	504	631	13.7
	17	1	7	5	8	3	37	92	261	390	13.4
	18	2	16	10	19	3	62	72	210	344	13.0
	19	1	44	9	10	8	76	74	147	297	11.4
	20	6	12	24	8	6	72	61	150	283	11.5
	21	1	5	38	9	12	88	99	205	392	11.5
	22	4	4	23	13	6	60	129	226	415	12.5
	23	1	38	31	23	0	97	82	171	350	13.1
	24	0	5	39	38	1	88	103	249	440	13.8
	25	0	15	30	29	0	79	55	233	367	12.4
	26	1	4	36	36	0	84	74	344	502	11.2
	27	2	9	24	3	0	55	52	96	205	9.1
	28	3	4	11	1	0	28	43	93*	164	7.0
	29	7	8	9	0	0	30	43	53	126	4.5
Total							1582	1869	5361	8712	

* Corrected for one missing day.

the figures obtained by adding the actual captures and second those by adding the logarithms of the numbers.

It will be seen that for the actual numbers, in seventeen out of the eighteen lunar months the captures of the Noctuidae in the "no moon" week were above those of the "full moon" week. The only exception is interesting as the 63 insects captured in that full moon week (October, 1933) was made up of no insects on each of five nights, one on one night, and 62 insects on the remaining night. This exceptional night (6 October) gave the highest total capture of Noctuidae of any night in the first two years and actually was a night of heavy cloud and therefore unlikely to be influenced by the moon. It will therefore be seen that the only exception to the rule of higher catches at no moon was statistically and meteorologically abnormal.

The second set of figures in Table III shows the same comparison based on the

TABLE III

Comparison of the Captures of Noctuidae in the Full Moon and No Moon Weeks in the Six Lunar Months of the Summers of 1933-1935. (Maximum of each pair in heavy type.) Also Similar Values for the Minimum Night Temperatures

Sum of numbers	May	June	July	Aug.	Sept.	Oct.	Years Total	All three years
1933—Week of full moon	15	55	58	64	29	63	284	
No moon	19	95	73	72	76	22	357	
1934—Full moon	2	52	37	69	61	1	222	1242
No moon	25	56	140	76	204	71	575	
1935—Full moon	2	15	477	168	64	10	736	2853
No moon	23	179	917	385	267	133	1904	
Sum of logarithms ($n + 1$)								
1933—Week of full moon	2.76	7.51	6.19	6.08	4.25	2.10	29.14	
No moon	3.59	7.36	7.15	7.14	7.34	3.89	36.46	
1934—Full moon	0.60	4.20	5.31	6.63	5.77	0.30	22.81	83.41
No moon	3.64	5.86	9.01	7.11	10.20	6.95	42.77	
1935—Full moon	0.60	2.75	10.87	8.99	6.32	1.93	31.46	133.20
No moon	3.49	7.93	13.86	11.70	9.92	7.07	53.97	
Mean minimum temperature								
1933—Week of full moon	48.3	53.0	58.7	56.6	52.3	44.1	52.2	
No moon	45.9	49.5	56.0	51.0	50.4	42.1	49.2	
1934—Full moon	41.1	48.3	51.4	51.7	48.0	48.0	48.1	49.1
No moon	41.6	49.0	53.7	54.3	51.4	46.6	49.4	
1935—Full moon	34.2	48.3	55.6	50.5	49.6	43.9	47.0	48.7
No moon	45.4	45.8	55.8	47.3	47.8	43.1	47.5	

sum of the logarithm of the catches each night. It will be seen that once more 17 out of the 18 comparisons give values in favour of no moon, and only one (that of June, 1933) slightly in favour of full moon. The comparison of the weeks in the lunar month in October, 1933, which gave a number value strongly in favour of full moon now gives a log value quite definitely in favour of full moon.

This indicates that the values in favour of no moon are due to consistent differences and not to occasional large and possibly accidental divergences.

The mean difference per week for the 18 weeks for the numbers is 87.6 ± 27.3 which gives a "*t*" test of significance of 3.2. On the logarithmic basis the mean difference is 2.77 ± 0.44 ; which gives "*t*" equal to 6.3, a value considerably more significant than that obtained from the numbers.

Before assuming that this apparent effect is due to the moon it is necessary to consider the possibility of other factors. It has been found in other investigations, to be published later, that the most important non-periodic single factor in determining the catch is the minimum temperature of the night. The last division in Table III shows the mean minimum temperatures for the same periods dealt with in the same manner as used for the summation of the Noctuidae. From this emerges the curious fact that in 1933 all of the six weeks of "no moon" were cooler than the corresponding week of "full moon"; while in 1934 the reverse occurred and five successive "no moon" weeks were warmer than the corresponding "full moon" weeks. In 1935 the temperatures were more regularly distributed.

The two sets of figures taken in conjunction show that in 1933 the differences in catch in favour of "no moon" were obtained in spite of an adverse temperature effect which alone would have tended to produce a preponderance in the opposite direction. The differences in 1934, are, on the contrary, in the same direction as those that might be expected from a temperature effect, with the exception of the last period which, curiously enough, gives the most striking example of a large difference in favour of no moon.

A combination of all the eighteen periods gives a very slight temperature effect in favour of the full moon (49.1°F. ; 48.7°F.) with a very definite capture in favour of the moonless nights (1242 : 2853 in total numbers and 83.4 : 133.2 in logs).

This preliminary investigation, neglecting the possible influence of cloud, which we have no reason to suppose is more prevalent at any particular phase of the moon, gives a very definite support to the idea that a real lunar effect is present.

Varying Effect Due to the Height of the Full Moon

It has been mentioned above that the full moons are high in the sky in December and low in June. It would therefore be expected that the full moon would have a greater effect in winter than in summer.

Unfortunately, practically no Noctuidae are captured in the winter months, but the Table IV shows average for the three years of the difference between the logs

of the captures at full moon and no moon weeks for each month from May to October.

TABLE IV

Variation in Difference of Lunar Effect in Different Summer Months Due to Height of Full Moon Above the Horizon

	May	June	July	August	Sept.	Oct.
Difference in week's totals	2.25	2.23	2.45	1.43	3.71	4.53
Mean difference per night	0.31	0.30	0.35	0.20	0.53	0.65

This is shown diagrammatically in fig. 1, and it will be seen that, with the exception of the month of August, the figures fit very closely to the expected results of low difference in June, gradually increasing towards the autumn. The low value for August is undoubtedly due to the fact that in two of the three years the full moons were warmer than the no moon periods in this month.

A daily difference of 0.31 in the logs as in May and June is equivalent to a doubling of the catch at no moon over full moon, while the daily difference of 0.65 in October is equivalent to an increase of over four times.

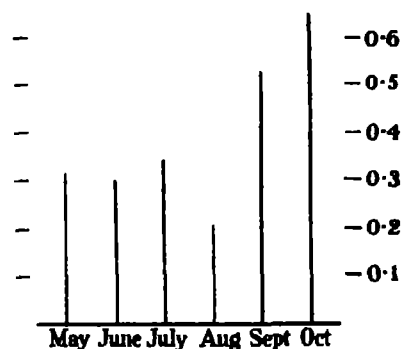


FIG. 1.—Diagram showing the difference in lunar effect on the Noctuidae in successive months, according to the height of the full moon.

Day-to-Day Departures of Logarithms from the 29-Day Mean

In order to extend the analysis to include all the days of the lunar period, the value for each night of the eighteen lunar months has been expressed as a departure of $\log(n+1)$ from the twenty-nine-day running mean. This, as has been explained, reduces the swamping effect of high individual catches and eliminates, at least partially, the effect of the rise and fall of the mean catch during the year.

Taking for granted the calculations, which are unnecessary to reproduce here, the figures in Table II now become as shown in Table V. Those for 1933 are given fully, but only the average figures for 1934 and 1935, and there is added a day-to-day mean departure for the three years together and finally the same smoothed to a five-day running mean. The results are shown diagrammatically in fig. 2 and to this has been added (dotted line) the mean minimum temperature calculated by the same method for comparison.

It will be seen that in each of the years there were large negative departures in captures about the time of full moon. In 1933 the positive departures are chiefly between no moon and the first quarter, while in 1934 they are from the last quarter to the first quarter (*i.e.*, the dark half of the cycle). In 1935 the highest positive departures are a few days after no moon. When the figures for the three years are

TABLE V

As Table II but with the Values for Each Day Expressed as Departures from the 29-Day Mean of the Logarithm of the Original Value

1933										1933-34-35		
Day in Lunar Month	Full Moon on							1934	1935	Five-day Smoothed		
	9 May	7 June	6 July	5 Aug.	3 Sept.	3 Oct.	Mean			Mean		
Full moon												
1	-0.38	+0.28	-0.01	-0.97	-0.07	-0.77	-0.32	-0.35	-0.42	-0.36	-0.28	
2	-0.37	+0.04	+0.03	+0.05	-0.32	-0.78	-0.23	-0.28	-0.60	-0.37	-0.28	
3	-0.07	+0.09	-0.13*	-0.34	-0.03	-0.45	-0.16	-0.42	-0.39	-0.32	-0.25	
4	+0.09	+0.33	-0.34	+0.01	-0.34	+1.06	+0.14	-0.35	-0.15	-0.12	-0.20	
5	+0.10	+0.17	-0.20*	-0.18	-0.34	+0.69	+0.04	-0.09	-0.21	-0.11	-0.14	
6	-0.11	-0.05	-0.12	+0.25	-0.43	-0.24	-0.12	-0.02	-0.12	-0.09	-0.10	
7	+0.30	+0.64	-0.22	-0.07	-0.41	-0.24	+0.0	-0.08	-0.03	-0.04	-0.06	
8	+0.07	+0.49	-0.46	+0.04	-0.71	-0.22	-0.13	-0.17	-0.08	-0.12	-0.03	
9	-0.11	+0.71	-0.60	+0.09	+0.0	-0.34	-0.04	+0.09	+0.04	+0.03	+0.02	
10	+0.45	-0.08	+0.18	+0.32	-0.17	-0.60	+0.02	+0.11	+0.10	+0.08	+0.06	
11	+0.22	-0.27	-0.10	+0.12	-0.31	+0.25	-0.02	+0.26	+0.19	+0.14	+0.09	
12	+0.11	-0.20	+0.12	+0.15	-0.31	-0.01	-0.02	+0.20	+0.26	+0.15	+0.13	
13	+0.09	-0.22	-0.04	-0.32	-0.05	+0.23	-0.05	+0.10	+0.13	+0.06	+0.13	
14	+0.54	-0.10	+0.30	+0.04	+0.21	-0.46	+0.10	+0.32	+0.21	+0.21	+0.14	
15	+0.04	-0.05	-0.01	+0.14	-0.06	-0.46	-0.07	+0.21	+0.19	+0.11	+0.15	
16	±0.0	-0.04	+0.32	-0.19	+0.07	+0.58	+0.12	+0.10	+0.28	+0.17	+0.16	
17	-0.20	-0.10	+0.62	-0.43	+0.04	+0.14	+0.01	-0.21	+0.38	+0.20	+0.15	
18	-0.04	+0.19	+0.24	-0.11	+0.43	+0.15	+0.14	+0.04	+0.39	+0.16	+0.16	
19	-0.24	+0.66	-0.18	-0.14	+0.18	+0.57	+0.14	+0.09	+0.13	+0.12	+0.16	
20	+0.22	+0.12	-0.03	+0.24	+0.38	+0.51	+0.24	+0.16	+0.08	+0.16	+0.15	
21	-0.28	-0.21	+0.50	+0.38	+0.07	+0.79	+0.21	+0.20	+0.12	+0.17	+0.14	
22	+0.09	-0.26	+0.15	+0.28	+0.22	+0.34	+0.13	+0.34	+0.02	+0.16	+0.13	
23	-0.33	+0.58	-0.21	+0.43	+0.46	-0.30	+0.11	+0.07	+0.13	+0.10	+0.10	
24	-0.68	-0.11	-0.34	+0.53	+0.68	+0.01	+0.02	+0.13	-0.02	+0.04	+0.07	
25	-0.68	+0.31	-0.16	+0.44	+0.58	-0.29	+0.03	-0.05	-0.13	+0.03	+0.05	
26	-0.38	-0.30	-0.18	+0.55	+0.70	-0.26	+0.04	+0.11	-0.10	+0.02	+0.0	
27	-0.22	+0.10	+0.25	+0.36	+0.77	-0.24	+0.17	+0.06	-0.11	+0.05	-0.05	
28	-0.10	-0.21	+0.28	+0.05	-0.25	-0.22	-0.08	-0.11	-0.17	-0.12	-0.12	
29	-0.20	+0.04	+0.04	-0.03	-0.54	-0.22	-0.23	-0.26	-0.13	-0.21	-0.20	
No moon												

* Trap not working : average value inserted.

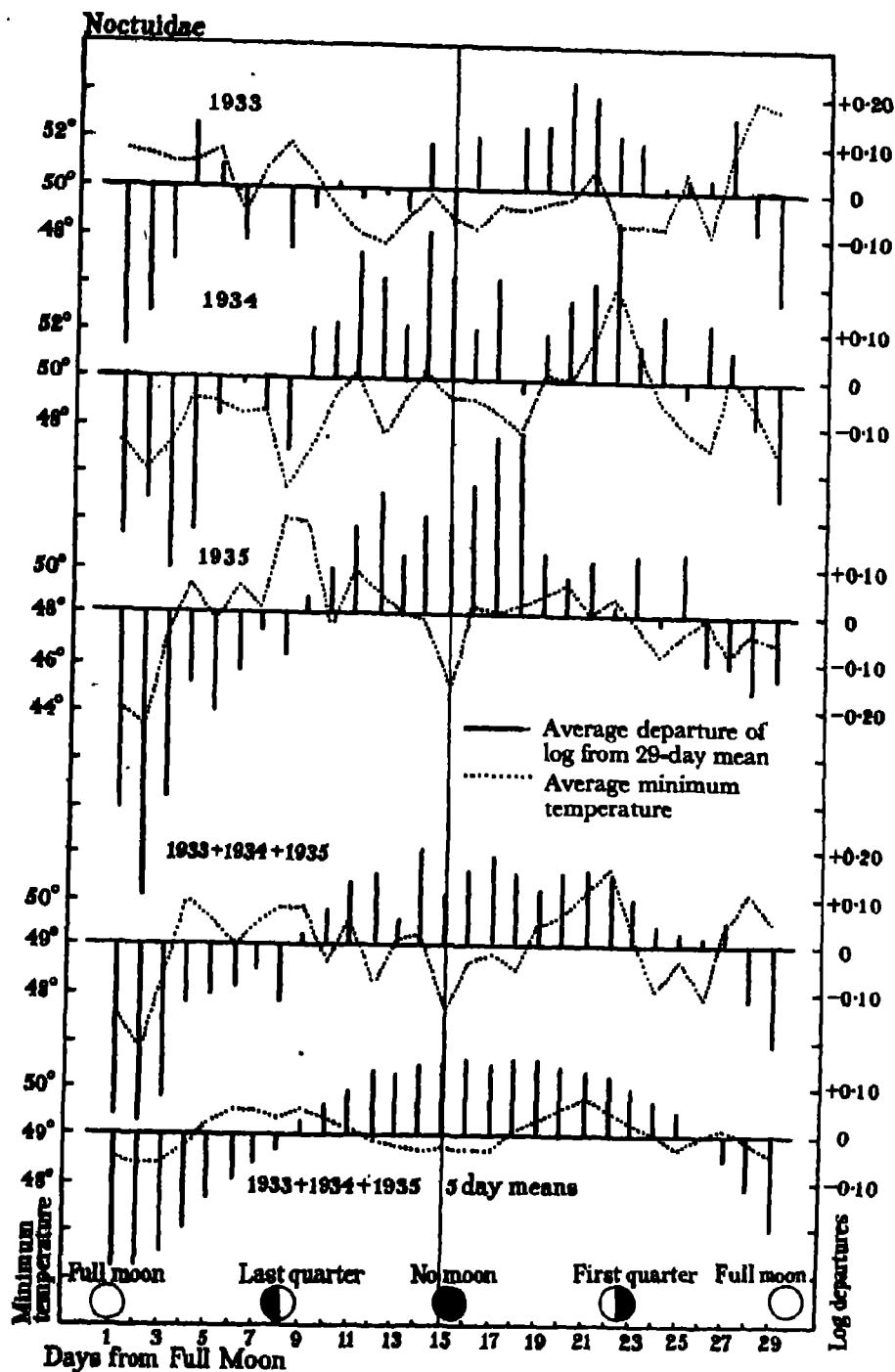


FIG. 2.—Diagram showing average departures from the 29-day mean catch of Noctuidae for successive days of the lunar month for six lunar months in the summers of 1933, 1934, and 1935, also (as dotted line) the mean departures of the minimum night temperatures.

combined and smoothed a fairly regular curve is produced giving the lowest captures at full moon ; the change over from negative to positive just after the last quarter ; then steadily increasing positive departures till about half-way between no moon and the first quarter ; and finally a rapid fall and the change-over from positive to negative between the first quarter and full moon..

The maximum positive departure in the five-day smoothed curve is 0.16 and the maximum negative departures — 0.28, giving a total difference of 0.44 which is equivalent to a catch at no moon of approximately 275 when the full moon catch is considered as 100.

Discussion on the Asymmetry of the Lunar Effect

The final curve in fig. 2 is asymmetrical in two ways : (a) the negative departure at the full moon period is shorter (11 days) and more extreme at its maximum (— 0.28) than the positive departure at the no moon period which lasts for 17 days and has a maximum departure of + 0.16 ;

(b) the positive departure continues high for a number of days after no moon, and the cross-over from positive to negative occurs only four days before full moon ; while the reverse change takes place 7–8 days after full moon.

The first asymmetry is almost certainly due to the fact that the intensity of the light of the moon (due to the proportion of its surface illuminated) and the duration of the moonlight through the night are both decreasing simultaneously as we pass from full moon towards new moon ; and at the first quarter not only has the reflected light been reduced to about half, but the duration of its effect during the night has also been reduced to half (on an average). This, with the reverse effect between no moon and full moon, would tend to give the curve a flat top and a narrower and more emphasized minimum.

The second asymmetry appears to be due to the irregularity of the sequence of the hours of setting and rising of the moon on successive days in the lunar month, which has already been mentioned briefly.

Figs. 3A, B, and C show diagrammatically the hours of rising and setting of the moon during a lunar month in May, in July, and in December-January. The figures are for the year 1934–5, but the shape of the curve is identical for the same date in all years.

The dark horizontal lines in the figure show the hours on successive days (from above and downwards) when the moon is below the horizon. Each dark line ends at moonrise and starts at moonset. The converging or diverging vertical lines show the times of the eight successive periods of the night into which the captures in the trap are divided, the centre line being at midnight. The times of sunset and sunrise are shown as broken vertical lines.

An examination shows that in May (fig. 3A), owing to the longer interval between successive risings before midnight, the first half of the night is already dark (*i.e.*, as regards moonlight) three to four days after full moon and well before the “last quarter” ; but owing to the rapidly shortening intervals between successive risings

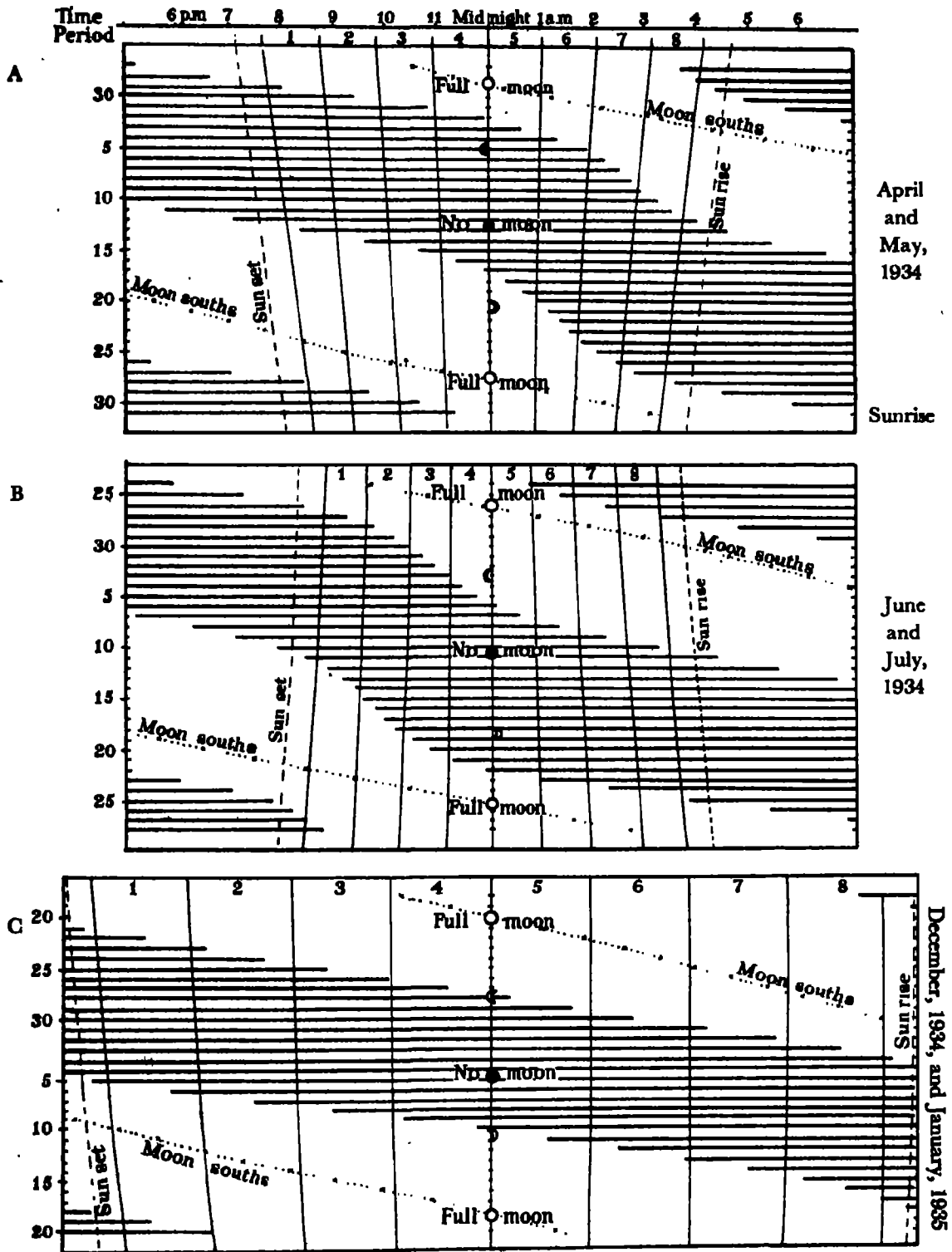


FIG. 3.—Diagram showing the hours of rising and setting of the moon at three different times of the year to show particularly the two types of asymmetry before and after mid-June and the symmetrical conditions in mid-winter.

it takes about ten days (the remainder of the half lunar month) for the second half of the night to become dark. A similar course follows with the setting of the moon after no moon; within four days of no moon the first half of the night is light, but ten more days are required for the second half to become light.

One might therefore expect, under these conditions, insects to be more abundant than average at the "last quarter" and less abundant at the "first quarter".

In July (fig. 3B), on the contrary, the reverse effect is found and it is not until about ten days after full moon that the first half of the night is dark, and not until ten days after no moon that the first half of the night is light. One might therefore expect fewer insects than normal at the last quarter and more at the first quarter.

Similar asymmetry continues throughout the autumn, but by mid-winter (fig. 3C) conditions have become practically symmetrical throughout the night. In the

TABLE VI

Mean Log Departures from the 29-Day Means for Each Day of the Lunar Months, as Table V, but Separated into the Periods Before and After mid-June to Show Differences in Asymmetry. Figures are Smoothed to Five-Day Running Means

Day of lunar month		May to Mid-June	Mid-June to October
Full Moon	1	- 0.15	- 0.34
	2	- 0.10	- 0.35
	3	- 0.08	- 0.33
	4	- 0.07	- 0.25
	5	- 0.03	- 0.16
	6	0.00	- 0.11
	7	+ 0.05	- 0.09
	8	+ 0.06	- 0.05
	9	+ 0.10	- 0.01
	10	+ 0.10	+ 0.04
	11	+ 0.13	+ 0.08
	12	+ 0.14	+ 0.12
	13	+ 0.13	+ 0.12
	14	+ 0.13	+ 0.12
No Moon	15	+ 0.11	+ 0.14
	16	+ 0.15	+ 0.16
	17	+ 0.08	+ 0.17
	18	+ 0.13	+ 0.17
	19	+ 0.08	+ 0.19
	20	+ 0.07	+ 0.18
	21	- 0.01	+ 0.19
	22	- 0.06	+ 0.19
	23	- 0.18	+ 0.18
	24	- 0.23	+ 0.16
	25	- 0.29	+ 0.11
	26	- 0.31	+ 0.05
	27	- 0.26	- 0.02
	28	- 0.23	- 0.14
	29	- 0.17	- 0.27

spring an asymmetry similar to that for May develops and persists till about mid-June, when there is a rapid change over to that of the July-autumn type.

The asymmetry that we have found to exist in the Noctuid moth captures is of the "autumn" type, but the figures were based on a summation of captures from

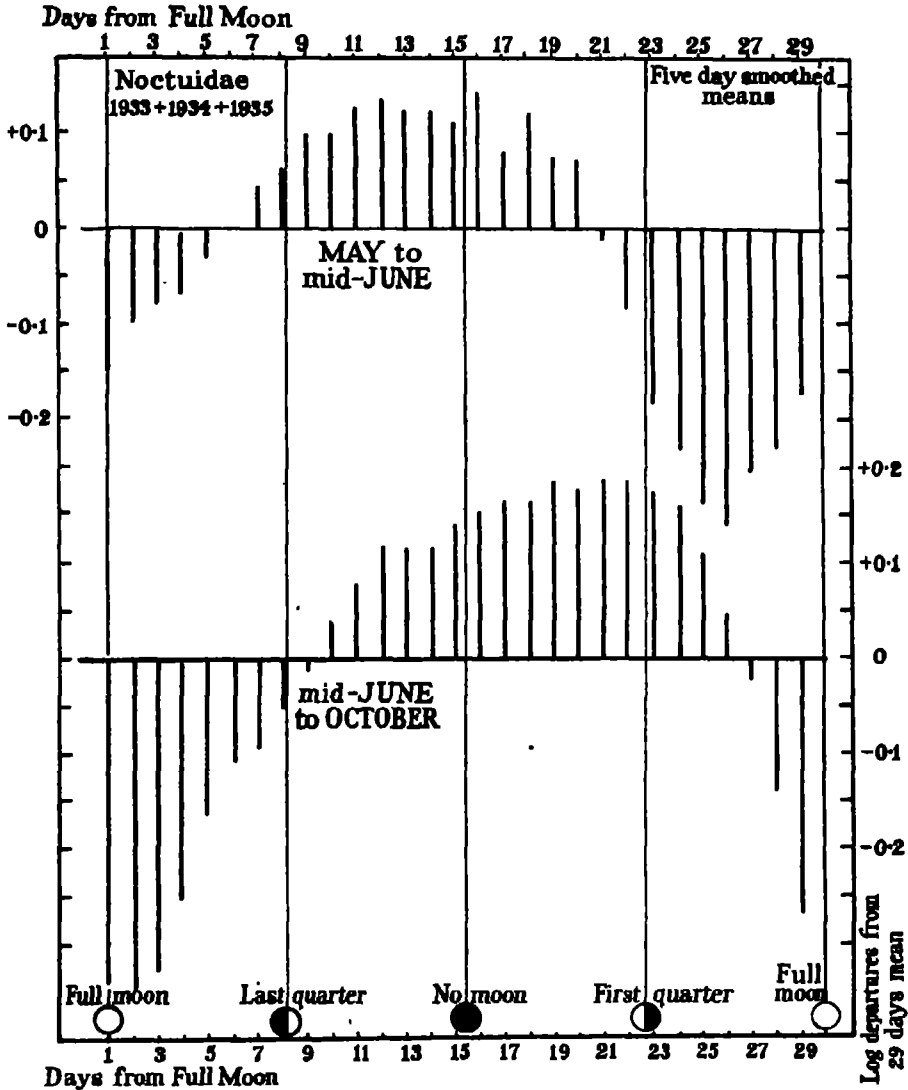


FIG. 4—Departures of the captures from the 29-day mean in the successive days of the lunar month separated into the periods before and after mid-June to show the two opposite types of asymmetry.

May to October. If the above lunar movements are the true cause of the insect asymmetry we should expect the Noctuid curve to show an asymmetry in one direction before mid-June and in the reverse direction after this time.

Table VI and fig. 4 show the results obtained when the captures of Noctuidae are treated as previously, after having been separated into two groups, (a) captures

from the beginning of May to mid-June ; and (b) captures from mid-June to the end of October. It will be seen that the results support the hypothesis, as the May curve shows higher catches at "last quarter" and lower at "first quarter" while the later curve shows the reverse. The only inexplicable feature at the moment is the very low captures about four or five days before full moon in the May-June curve; but as this is based on a relatively small number of figures it may be accidental.

The original asymmetry of the curve in fig. 2 is therefore only due to the fact that it was based on a larger number of observations after mid-June than before.

Effect of the Phase of the Moon on the Time of Flight of the Noctuidae

Table VII and fig. 5 (histogram) show the normal distribution of the Noctuidae through the eight equal periods of the night into which the trap separates the catches. This is based on all captures in the summers of 1933, 1934, and 1935. It will be seen that the maximum flight is just after midnight in period 5, if calculated on either a number or logarithmic basis, but that almost equal numbers come before and after midnight (47% to 53%).

If the moon is inhibiting activity it would be expected that the catch in the early portion of the night would be reduced when the moon was above the horizon early (*i.e.*, before full moon) ; and that the catch in the later portion of the night would be reduced when the moon was late (*i.e.*, after the full moon). To test this the night distribution was calculated separately for the weeks before full moon and after full moon for the three summers under discussion. Each set of figures in Table VII thus represents the results of eighteen weeks' captures. The night of full moon is omitted.

TABLE VII

Numbers of Noctuidae Captured in Each Period of the Night for All Nights ; for Nights in the Week before Full Moon ; and for Nights in the Week after Full Moon

Period of Night :	1	2	3	4	5	6	7	8
<i>Actual numbers</i>								
All captures	599	828	1013	1454	1759	1429	955	309
Weeks before full moon	115	174	175	362	467	380	230	52
Weeks after full moon	75	136	150	234	190	152	70	35
<i>Sum of logarithm (n + 1)</i>								
All captures	99.4	140.8	161.7	182.7	185.6	168.4	121.4	55.8
Weeks before full moon	21.1	27.8	30.7	38.9	43.0	39.3	30.0	13.1
Weeks after full moon	16.8	26.9	27.0	32.8	29.0	27.2	16.6	8.2
% (actual numbers)	Before midnight				After midnight			
All captures	47				53			
Before full moon	42				58			
After full moon	57				43			

Fig. 5 shows the results diagrammatically superimposed on the normal distribution, the vertical scale for the separate weeks being four times that of the total (histogram).

It will be seen that the results are according to expectation. There is a slight but distinct shift of the distribution later, in the week before full-moon, when there is still darkness in the second half of the night ; and a corresponding shift earlier, in the week after full moon when the darkness is gradually increasing in the first half of the night.

In the week before full moon 58% of the catches are after midnight and 42 before, while in the week after no moon 57% are before midnight and 43 after.

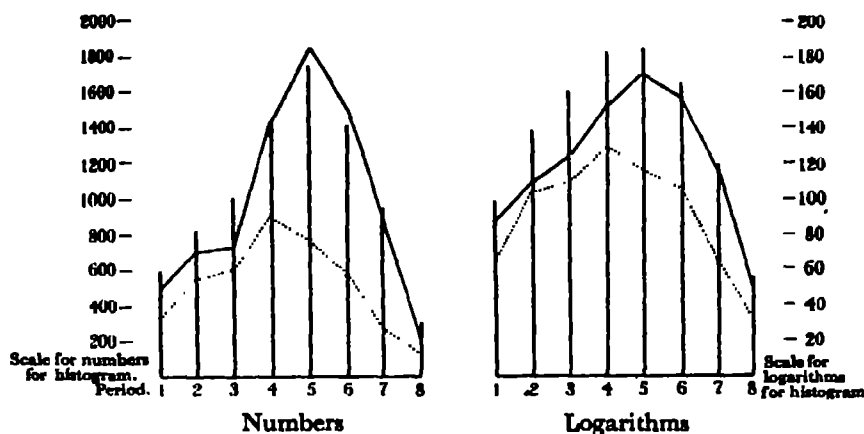


FIG. 5—Diagram showing the normal distribution of the Noctuidae in the eight periods of the night (histogram) and also the distribution in the week before full moon (continued line) and in the week after full moon (dotted line).

Interrelation of the Influence of Cloud and Moonlight

Up till now the figures have been analysed to show the presence of a lunar effect irrespective of the state of the sky.

To show the possible influence of cloud it was decided to divide the days in the lunar months under review in two ways.

- (1) As regards the state of the moon, into three divisions :—
 - (a) nights during the week of full moon ;
 - (b) nights during the weeks of intermediate moon ;
 - (c) nights during the week of no moon.
- (2) As regards the state of the sky, into three divisions :—
 - (a) nights with more than 90% of the sky clear ("clear") ;
 - (b) nights with 10–90% of the sky cloudy ("intermediate") ;
 - (c) nights with more than 90% cloudy ("cloudy").

The interrelation of these two main divisions gives nine possible combinations as shown in Table VIII.

The next stage was to allot each night of the six lunar months in each year into its proper division. Table VIII shows the actual results for 1933 as an example of the method. It will be seen that the numbers of nights in each division are different, depending entirely on accidental weather conditions.

TABLE VIII

Nights in 1933 Divided Into Nine Groups According to the Combination of Conditions of Moon and Cloud

	Clear	Intermediate	Cloudy
Full moon week	June : 4, 5, 6, 7, 8. July : 3, 4, 7. August : 3, 4, 5, 7, 8. Sept. : 2, 4, 5, 6. Oct. : 4.	May : 10, 11, 12. June : 9. July : 6. August : 2, 3. Oct. : 2, 3, 5, 6*.	May : 9. June : 10. July : 5, 9*. August : 31. Sept. : 1, 3, 30. Oct. : 1, 30.
First and third quarters	May : 18, 19. June : 1, 2, 3, 28. July : 2, 14, 16, 25, 26, 27. August : 1, 12, 16, 26, 27, 28. Sept. : 7, 8, 9, 10, 14. Oct. : 11, 12, 14, 25*, 27.	May : 14, 15, 17, 20, 29, 30, 31. June : 11, 13, 14, 17, 18, 26, 27, 30. July : 1, 11, 12, 29. August : 9, 10, 13, 14, 24, 25, 29, 30. Sept. : 13, 22, 25. Oct. : 8, 23, 26*.	May : 13, 16, 28. June : 12, 15, 16, 29. July : 13, 15, 17, 28, 29, 31*. August : 11, 15. Sept. : 11, 12, 23, 24, 28, 27, 28, 29. Oct. : 7, 9*, 10*, 13, 22, 24*, 28, 29.
No moon week	May : 21, 22. June : 19, 21. July : 18, 19, 20. August : 23. Sept. : 15. Oct. : 15, 16*, 17, 19.	May : 23, 26, 27. June : 22, 23. July : 21, 22. August : 17, 18, 19, 20, 21. Sept. : 16, 18, 19, 20, 21. Oct. : 20.	May : 24, 25. June : 20, 24, 25. July : 23, 24. August : 22. Sept. : 17. Oct. : 18, 21.

* = nights with heavy wind.

Certain of these days, marked with an asterisk, had captures considerably reduced by heavy wind. These have been eliminated from further calculations.

In each of the subdivisions the departures of the logs from the 29-day mean for the correct days were placed and these were summed and divided by the number of days, thus giving an average departure for that particular combination of cloud and moon conditions.

Similarly averages were worked out for each row and column, giving the effect of moon independent of cloud and cloud independent of moon respectively.

Table IV shows the results thus obtained for the Noctuidae in the years 1933, 1934, and 1935 (*a*, *b*, and *c*) separately and also for the three combined together (*g*).

TABLE IX

Analysis of Noctuid Captures and Minimum Temperatures During 1933-35 According to Conditions of Moon and Cloud

Mean log : departures 1933				1934				1935					
a	{	-0.155	-0.064	+0.038	-0.089	-0.394	-0.239	-0.023	-0.268	-0.437	-0.360	-0.059	-0.232
		-0.125	+0.097	+0.193	+0.056	+0.044	+0.144	+0.051	+0.099	+0.001	+0.083	+0.113	+0.060
		+0.029	+0.051	+0.144	+0.074	+0.133	+0.149	+0.204	+0.159	+0.110	+0.243	+0.332	+0.207
		-0.102	+0.053	+0.171		-0.064	+0.072	+0.087		-0.086	-0.015	+0.139	
Mean minimum temperatures: departures													
1933													
d	{	+2.24	-0.72	+3.20	+1.6	-3.0	+0.16	+2.6	-0.86	-3.16	-1.48	+0.5	-0.93
		-2.34	-1.16	+1.61	-0.68	-1.8	+0.63	+2.1	+0.18	-1.31	+0.72	+3.37	+0.70
		-2.50	-1.43	+1.33	-1.0	-0.57	-0.14	+4.1	+0.83	-4.37	+0.66	+2.03	-0.53
		-0.92	-0.80	+1.9		-1.9	+3.9	+2.9		-2.41	+0.09	+2.35	
1934													
f	{												
1935													
i	{												
Mean catch (anti-log -1)													
1933 + 1934 + 1935													
j	{	-0.307	-0.254	-0.019	-0.219	0.575	0.628	0.863	0.663	2.76	3.25	6.30	3.60
		-0.028	+0.111	+0.133	+0.071	0.854	0.993	1.015	0.953	6.14	8.84	9.35	7.97
		+0.095	+0.141	+0.238	+0.147	0.977	1.023	1.120	1.029	8.48	9.54	12.18	9.69
		-0.079	+0.037	+0.129		0.803	0.919	1.011		5.35	7.30	9.26	
Mean minimum temperature: departures													
1933 + 1934 + 1935													
k	{	42	49	54		-0.94	-0.78	+2.26	-0.07				
		92	133	120		-1.79	+0.17	+2.43	+0.08				
		128	144	146		-2.43	-0.42	+2.44	-0.23				
		80	110	140		-1.71	-0.07	+2.32					
Mean minimum temperature: departures													
1933 + 1934 + 1935													
l	{	42	49	54		-0.94	-0.78	+2.26	-0.07				
		92	133	120		-1.79	+0.17	+2.43	+0.08				
		128	144	146		-2.43	-0.42	+2.44	-0.23				
		80	110	140		-1.71	-0.07	+2.32					
Mean minimum temperature: departures													
1933 + 1934 + 1935													
m	{	42	49	54		-0.94	-0.78	+2.26	-0.07				
		92	133	120		-1.79	+0.17	+2.43	+0.08				
		128	144	146		-2.43	-0.42	+2.44	-0.23				
		80	110	140		-1.71	-0.07	+2.32					
Mean minimum temperature: departures													
1933 + 1934 + 1935													
n	{	42	49	54		-0.94	-0.78	+2.26	-0.07				
		92	133	120		-1.79	+0.17	+2.43	+0.08				
		128	144	146		-2.43	-0.42	+2.44	-0.23				
		80	110	140		-1.71	-0.07	+2.32					
Mean minimum temperature: departures													
1933 + 1934 + 1935													
o	{	42	49	54		-0.94	-0.78	+2.26	-0.07				
		92	133	120		-1.79	+0.17	+2.43	+0.08				
		128	144	146		-2.43	-0.42	+2.44	-0.23				
		80	110	140		-1.71	-0.07	+2.32					
Mean minimum temperature: departures													
1933 + 1934 + 1935													
p	{	42	49	54		-0.94	-0.78	+2.26	-0.07				
		92	133	120		-1.79	+0.17	+2.43	+0.08				
		128	144	146		-2.43	-0.42	+2.44	-0.23				
		80	110	140		-1.71	-0.07	+2.32					
Mean minimum temperature: departures													
1933 + 1934 + 1935													
q	{	42	49	54		-0.94	-0.78	+2.26	-0.07				
		92	133	120		-1.79	+0.17	+2.43	+0.08				
		128	144	146		-2.43	-0.42	+2.44	-0.23				
		80	110	140		-1.71	-0.07	+2.32					
Mean minimum temperature: departures													
1933 + 1934 + 1935													
r	{	42	49	54		-0.94	-0.78	+2.26	-0.07				
		92	133	120		-1.79	+0.17	+2.43	+0.08				
		128	144	146		-2.43	-0.42	+2.44	-0.23				
		80	110	140		-1.71	-0.07	+2.32					
Mean minimum temperature: departures													
1933 + 1934 + 1935													
s	{	42	49	54		-0.94	-0.78	+2.26	-0.07				
		92	133	120		-1.79	+0.17	+2.43	+0.08				
		128	144	146		-2.43	-0.42	+2.44	-0.23				
		80	110	140		-1.71	-0.07	+2.32					
Mean minimum temperature: departures													
1933 + 1934 + 1935													
t	{	42	49	54		-0.94	-0.78	+2.26	-0.07				
		92	133	120		-1.79	+0.17	+2.43	+0.08				
		128	144	146		-2.43	-0.42	+2.44	-0.23				
		80	110	140		-1.71	-0.07	+2.32					
Mean minimum temperature: departures													
1933 + 1934 + 1935													
u	{	42	49	54		-0.94	-0.78	+2.26	-0.07				
		92	133	120		-1.79	+0.17	+2.43	+0.08				
		128	144	146		-2.43	-0.42	+2.44	-0.23				
		80	110	140		-1.71	-0.07	+2.32					
Mean minimum temperature: departures													
1933 + 1934 + 1935													
v	{	42	49	54		-0.94	-0.78	+2.26	-0.07				
		92	133	120		-1.79	+0.17	+2.43	+0.08				
		128	144	146		-2.43	-0.42	+2.44	-0.23				
		80	110	140		-1.71	-0.07	+2.32					
Mean minimum temperature: departures													
1933 + 1934 + 1935													
w	{	42	49	54		-0.94	-0.78	+2.26	-0.07				
		92	133	120		-1.79	+0.17	+2.43	+0.08				
		128	144	146		-2.43	-0.42	+2.44	-0.23				
		80	110	140		-1.71	-0.07	+2.32					
Mean minimum temperature: departures													
1933 + 1934 + 1935													
x	{	42	49	54		-0.94	-0.78	+2.26	-0.07				
		92	133	120		-1.79	+0.17	+2.43	+0.08				
		128	144	146		-2.43	-0.42	+2.44	-0.23				
		80	110	140		-1.71	-0.07	+2.32					
Mean minimum temperature: departures													
1933 + 1934 + 1935													
y	{	42	49	54		-0.94	-0.78	+2.26	-0.07				
		92	133	120		-1.79	+0.17	+2.43	+0.08				
		128	144	146		-2.43	-0.42	+2.44	-0.23				
		80	110	140		-1.71	-0.07	+2.32					
Mean minimum temperature: departures													
1933 + 1934 + 1935													
z	{	42	49	54		-0.94	-0.78	+2.26	-0.07				
		92	133	120		-1.79	+0.17	+2.43	+0.08				
		128	144	146		-2.43	-0.42	+2.44	-0.23				
		80	110	140		-1.71	-0.07	+2.32					
Mean minimum temperature: departures													
1933 + 1934 + 1935													
aa	{	42	49	54		-0.94	-0.78	+2.26	-0.07				
		92	133	120		-1.79	+0.17	+2.43	+0.08				
		128	144	146		-2.43	-0.42	+2.44	-0.23				
		80	110	140		-1.71	-0.07	+2.32					
Mean minimum temperature: departures													
1933 + 1934 + 1935													
ab	{	42	49	54		-0.94	-0.78	+2.26	-0.07				
		92	133	120		-1.79	+0.17	+2.43	+0.08				
		128	144	146		-2.43	-0.42	+2.44	-0.23				
		80	110	140		-1.71	-0.07	+2.32					
Mean minimum temperature: departures													
1933 + 1934 + 1935													
ac	{	42	49	54		-0.94	-0.78	+2.26	-0.07				
		92	133	120		-1.79	+0.17	+2.43	+0.08				
		128	144	146		-2.43	-0.42	+2.44	-0.23				
		80	110	140		-1.71	-0.07	+2.32					
Mean minimum temperature: departures													
1933 + 1934 + 1935													
ad	{	42	49	54		-0.94	-0.78	+2.26	-0.07				
		92	133	120		-1.79	+0.17	+2.43	+0.08				
		128	144	146		-2.43	-0.42	+2.44	-0.23				
		80	110	140		-1.71	-0.07	+2.32					
Mean minimum temperature: departures													
1933 + 1934 + 1935													
ae	{	42	49	54		-0.94	-0.78	+2.26	-0.07				
		92	133	120		-1.79	+0.17	+2.43	+0.08				
		128	144	146		-2.43	-0.42	+2.44	-0.23				
		80	110	140		-1.71	-0.07	+2.32					
Mean minimum temperature: departures													
1933 + 1934 + 1935													
af	{	42	49	54		-0.94	-0.78	+2.26	-0.07				
		92	133	120		-1.79	+0.17	+2.43	+0.08				
		128	144	146		-2.43	-0.42	+2.44	-0.23				
		80	110	140		-1.71	-0.07	+2.32					
Mean minimum temperature: departures													
1933 + 1934 + 1935													
ag	{	42	49	54		-0.94	-0.78	+2.26	-0.07				
		92	133	120		-1.79	+0.17	+2.43	+0.08				
		128	144	146		-2.43	-0.42	+2.44	-0.23				
		80	110	140		-1.71	-0.07	+2.32					
Mean minimum temperature: departures													
1933 + 1934 + 1935													
ah	{	42	49	54		-0.94	-0.78	+2.26	-0.07				
		92	133	120		-1.79	+0.17	+2.43	+0.08				
		128	144	146		-2.43	-0.42	+2.44	-0.23				
		80	110	140		-1.71	-0.07	+2.32					
Mean minimum temperature: departures													
1933 + 1934 + 1935													
ai	{	42	49	54		-0.94	-0.78	+2.26	-0.07				
		92	133	120		-1.79	+0.17	+2.43	+0.08				
		128	144	146		-2.43	-0.42	+2.44	-0.23				
		80	110	140		-1.71	-0.07	+2.32					
Mean minimum temperature: departures													
1933 + 1934 + 1935													
aj	{	42	49	54		-0.94	-0.78	+2.26	-0.07				
		92	133	120		-1.79	+0.17	+2.43	+0.08				
		128	144	146		-2.43	-0.42	+2.44	-0.23				
		80	110	140		-1.71	-0.07	+2.32					
Mean minimum temperature: departures													
1933 + 1934 + 1935													
ak	{	42	49	54		-0.94	-0.78	+2.26	-0.07				
		92	133	120		-1.79	+0						

It will be seen that in general there is a tendency for the "clear" column to be lower than the "cloudy", and for the "full moon" to be lower than the "no moon" row. The division "full moon—clear sky" gives in each case the greatest negative departure, and the division "no moon—cloudy" gives the greatest positive departures.

Since the mean logarithms for the whole of the captures of the series is known to be 0.882 (equal to a geometric mean catch of 6.62 insects) the mean departures for the three years (Table IXg) can be subtracted from or added to this mean and a true logarithm (*h*) and hence an average catch in numbers can be obtained for each division. This is shown in Table IXi with the final addition of a Table IXj in which the figures are altered to bring the mean catch to 100, so that the figures in each division are percentages of the mean catch.

This table is consistent except for the figure 133 in the "intermediate cloud, intermediate moon" division which is a little higher than might be expected. The figures for the rows and columns separately are quite consistent and show a ratio of 146 : 120 : 54 for nights with no moon, intermediate moon, and full moon respectively; and a ratio 140 : 110 : 80 for nights of full cloud, intermediate cloud, and clear sky. The ratio of catches in the extreme conditions of "no moon—cloudy" and "full moon—clear" is 184 : 42 or just over 4 : 1.

Another point worthy of notice is that the reduction of catch due to the clearness of the sky is greater on full moon nights (95 : 42) than on moonless nights (184 : 128); and similarly the reduction of catch by moonlight is greater on clear nights (128 : 42) than on cloudy nights (184 : 95).

A three-dimensional model of the nine main values in Table IXj is shown in fig. 6 with the full moon row in front and the clear sky row to the right. The height of the vertical columns represents the catch under each combination of conditions.

These figures must next be considered in connexion with possible temperature variations, and for this purpose the minimum temperatures have been treated in the same way as the figures for captures (except that they are not converted to logarithms) and the results are shown in Table IXd, e, f, and k.

It will be seen that in each year there is a consistent temperature gradient from cloudy nights to clear which is to be expected owing to the action of clouds in preventing radiation. On the average of the three years the difference is 4.03° F., which is possibly sufficient to explain the differences in captures in favour of cloudy nights. A fuller investigation of this will be made later.

The temperature in relation to moon phase shows (as has already been mentioned) warmer conditions at full moon in 1933; at no moon in 1934; and at intermediate moon in 1935.

On the three years combined there is still a bias of about 0.3° F. in favour of the full moon, so that the results obtained are not due to any accidental temperature effect, and, in fact, if temperature had been normally distributed the effect of the moon might be expected to be slightly greater than that shown.

It will also be seen that the temperature departure in the division "intermediate moon—intermediate cloud" is considerably less than that in "no moon—intermediate cloud", so that the inconsistency of the catch figures for the former division is at least partly explained.

To sum up—the ratio of catches in the Noctuidae in the three years combined is shown to be about 2.7 : 1 when no moon is compared to full moon (in spite of a small temperature effect in favour of full moon) ; and 1.75 : 1 when cloudy nights are compared with clear nights, this being associated with a temperature difference of about 4° F. in favour of the cloudy nights (owing to reduction of radiation by the clouds), and possibly explicable on this basis alone. Finally the ratio between

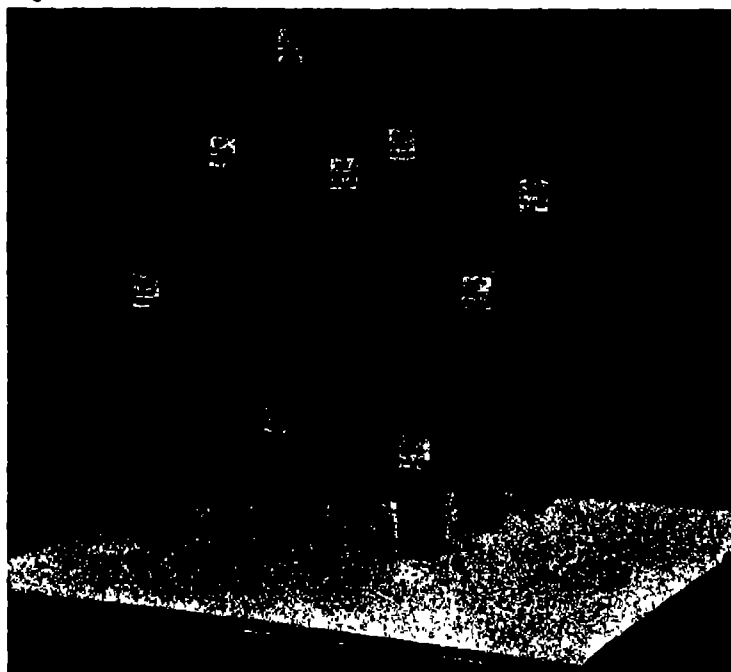


FIG. 6—Photograph of three-dimensional model showing the average numbers of Noctuidae captured in the trap on nights of various combinations of moon phase and cloud conditions.

"no moon—cloudy" and "full moon—clear" is just over 4 : 1, while in the cross-relation, clear nights with no moon give distinctly larger catches than cloudy nights with full moon (128 : 95). It must, however, be recollected that the group "cloudy" nights includes any night with less than 10% of the sky clear, so that slight lunar influence could be expected on some of these nights, particularly as the light of the moon can penetrate clouds of considerable thickness.

Correction of the 29-Day Mean Curve for Lunar Influence

In fig. 7 are shown as vertical columns the log of the catches (+.1) of the Noctuidae each day during the six lunar months of the three years under consideration. There

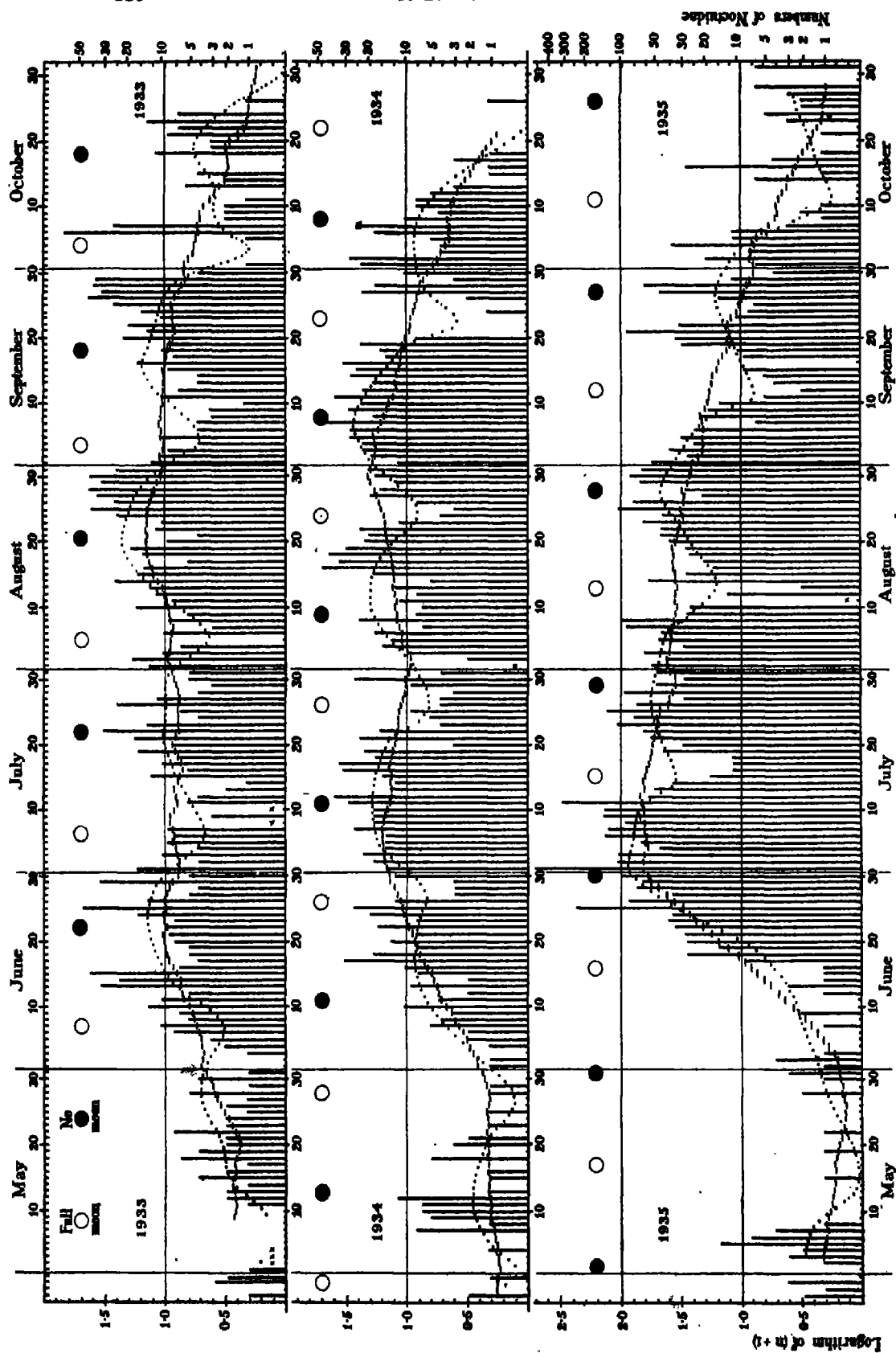


Fig. 7.—The captures of Noctuidae during the summer months of 1933-35 (represented as $\log(n+1)$) together with the 29-day running mean of their values, and the same mean (dotted line) corrected to eliminate the lunar influences.

is also shown as a series of short horizontal lines the running 29-day mean of these values. The difference between the value for any one day and the 29-day mean for the same day is due to the conditions of that day, including such non-periodic factors as wind, temperature, cloud, and humidity, which change rapidly from day to day, and also to the phase of the moon which is not eliminated by taking the mean over the same period as the length of the lunar months.

With the information we have gained above, however, it is now possible to correct this 29-day mean for the expected lunar influence based primarily on the curves shown in figs. 4 and 6 and the information in Table IV.

The factors that have to be considered are

- (1) the mean + and - departures at no moon and full moon ;
- (2) the fact that the differences are at their minimum in June and maximum in October ;
- (3) the alteration in asymmetry of the corrections from the months before mid-June to those after.

With these facts as a basis, there has finally been added to fig. 7 a third curve (dotted) showing the 29-day mean corrected as nearly as possible for the lunar influence.

It will immediately be seen how much more closely the curve fits the changes in the log histogram, than did the 29-day mean ; thereby showing what a high proportion of the variation is due to the lunar influence.

For example, the log ($n + 1$) value for 25 July, 1934, is about 0.1 below the 29-day mean, but actually about 0.15 above the corrected mean. Thus the more rapidly changing weather conditions such as temperature, etc., are actually responsible for an increase in the captures above normal and not a decrease as would appear if the lunar effect were neglected.

From this figure, therefore, it is possible to start with a new set of values for each day showing departures from which the lunar periodicity has been practically eliminated, and from which the influence of other factors can now be studied with considerable simplification due to the elimination of a periodic change.

LUNAR INFLUENCE ON OTHER GROUPS OF INSECTS

Comparison of Full Moon and No Moon Weeks in Various Groups

Table X shows the comparison of full and no moon week captures, calculated in a way similar to that shown for the Noctuidae in Table III, for a number of other groups of insects into which the night catches were sorted. They are all based on the logarithm of the numbers to eliminate as far as possible chance high catches.

It will be seen first of all that in 1934 there are very few exceptions to the rule that catches are highest in no moon weeks. In 1935, there are several exceptions, particularly in the month of August ; while in 1933 the exceptions are quite numerous

TABLE X
Difference in Captures in Weeks of Full Moon and No Moon in Various Groups of Insects for Comparison with Table III

	1933							1934							1935							All years
	Total							Total							Total							
All insects	15.5	18.7	16.8	19.0	16.4	10.3	96.7	8.7	14.8	16.0	16.1	14.3	11.8	81.6	7.6	16.9	21.5	19.7	15.3	11.9	93.0	271.2
	20.8	17.3	23.0	12.1	14.5	10.8	98.5	9.3	17.8	20.0	17.0	16.8	17.4	98.2	17.2	19.3	25.5	17.3	17.7	17.5	114.4	311.1
All Lepidoptera	9.3	14.0	15.5	13.1	11.2	3.7	66.7	3.2	11.1	13.7	12.4	7.6	1.4	49.4	1.6	10.6	17.8	14.7	8.2	2.7	55.6	171.7
	10.7	13.7	18.4	8.7	9.7	3.9	65.2	5.0	13.7	17.6	13.1	11.6	7.7	68.8	8.2	12.2	20.2	14.5	10.8	7.5	73.3	208.3
Geometridae	3.6	6.2	6.9	5.6	2.4	1.1	25.7	1.3	3.9	2.2	3.3	0.8	0.0	11.5	0.0	3.6	7.0	5.7	1.4	0.8	18.5	55.7
	8.3	4.9	11.1	1.2	0.6	1.5	27.5	1.3	6.9	8.6	5.4	3.3	2.3	27.8	2.6	3.9	6.6	5.2	2.6	1.2	22.1	77.3
Crambidae	2.7	9.2	12.5	5.7	0.3	0.0	30.5	0.0	6.2	12.4	9.6	1.6	0.0	29.8	0.0	7.9	15.8	12.3	0.0	0.0	36.0	96.3
	0.6	10.4	15.8	3.2	0.0	0.0	30.0	0.0	9.9	14.8	10.4	2.3	0.0	37.3	0.0	7.4	19.2	10.5	3.2	0.0	40.3	107.6
Colcoptera	2.0	4.6	5.6	5.2	0.5	0.0	17.9	2.3	3.6	1.7	2.6	2.0	1.3	13.5	1.1	1.2	7.1	4.0	1.0	0.9	15.4	46.8
	2.7	1.4	7.0	1.6	0.8	0.6	14.1	3.7	4.2	4.1	3.5	3.8	2.3	21.6	2.8	1.6	4.8	2.8	2.0	1.4	15.4	51.1
Psocoptera	0.0	1.4	3.0	5.5	5.5	0.8	16.1	0.0	0.0	2.3	1.5	3.9	0.3	8.0	0.0	0.0	0.3	0.0	0.0	0.3	0.6	24.7
	0.0	0.6	4.4	1.5	2.3	0.3	9.1	0.0	0.9	0.0	2.7	8.3	2.4	14.2	0.0	0.0	0.0	0.0	0.0	0.5	0.5	23.9
Jassidae	1.0	6.6	9.6	10.5	5.9	0.8	34.8	0.0	1.2	2.0	0.9	2.1	0.0	6.2	0.0	0.0	4.9	6.7	1.9	0.3	13.8	54.8
	0.0	2.6	7.2	0.8	1.9	0.7	13.1	0.3	0.8	7.0	4.1	7.6	5.5	25.2	0.0	1.0	3.0	2.2	2.1	3.5	11.7	50.3
Aphididae	3.0	6.2	9.0	10.1	6.1	1.5	35.8	0.0	1.1	2.0	0.9	0.8	0.6	5.3	0.0	0.3	2.0	4.0	0.6	0.3	7.2	48.3
	0.9	2.5	9.8	2.6	1.2	0.6	17.7	0.0	0.8	2.3	1.8	3.2	1.1	9.1	1.1	1.3	0.6	2.5	1.1	0.6	7.1	34.0

	1933					1934					1935					All					
Capsidae	Total					Total					Total					Years					
0-0	2-3	6-3	2-8	0-0	0-0	11-4	0-0	1-2	2-0	0-9	1-8	0-0	5-9	0-0	0-0	3-1	0-0	8-1	25-4		
0-0	0-3	7-4	0-0	0-8	0-3	8-7	0-3	0-8	7-4	4-1	7-6	0-3	20-4	0-3	0-0	4-2	1-6	1-4	0-0	7-4	36-6
Borboridae																					
2-1	2-7	0-0	2-8	3-8	1-2	12-5	0-7	1-4	4-3	8-6	3-3	1-3	19-5	2-8	8-7	2-7	12-7	3-4	4-5	34-9	66-8
2-6	4-6	3-4	1-1	1-5	1-6	14-5	1-3	2-5	0-0	8-3	5-0	6-7	23-7	4-5	6-7	8-5	5-6	12-0	8-2	45-6	83-7
Cecidomyiidae																					
4-0	10-1	14-0	13-6	8-1	1-6	51-4	0-0	6-2	7-6	8-2	7-8	0-0	29-7	0-0	4-7	15-9	14-7	8-4	0-7	44-4	125-5
8-1	9-7	18-7	5-0	6-0	0-0	47-5	1-4	9-4	13-7	12-1	8-3	3-0	47-9	4-0	6-9	15-2	10-1	5-5	4-9	46-4	141-8
Ceratopogon																					
7-3	9-5	11-9	11-3	4-5	3-2	47-8	2-9	5-1	3-9	7-7	7-4	2-8	29-8	2-8	4-5	8-3	10-9	6-5	6-2	39-3	116-8
14-3	10-5	17-9	3-3	7-2	2-4	55-6	4-1	5-4	7-3	8-6	8-4	10-0	46-8	7-1	5-6	6-6	7-7	9-3	8-2	44-3	143-7
Chironomidae (excluding Ceratopogon)																					
7-0	11-2	11-8	12-1	11-6	6-3	60-1	5-2	8-0	9-6	10-4	7-8	7-5	48-5	1-7	11-1	13-4	11-7	13-1	7-1	58-1	166-6
9-0	10-2	14-2	6-4	9-6	0-6	49-9	3-9	14-5	12-3	7-9	11-8	10-4	59-9	10-3	10-7	13-0	8-0	10-6	10-9	63-4	173-2
Psychodidae																					
11-4	14-5	11-4	10-4	7-7	2-0	57-3	2-9	7-4	5-5	7-1	4-9	2-2	30-0	2-2	12-2	15-6	13-2	7-9	4-2	55-3	142-6
19-0	11-2	16-8	2-1	4-5	0-8	54-4	2-4	11-8	13-8	6-4	5-7	9-1	49-0	14-9	15-3	20-8	5-8	4-4	6-9	68-2	171-6
Mycetophyllidae (chiefly Sciara)																					
3-4	6-2	7-1	5-9	4-7	0-5	27-7	0-0	1-8	2-7	2-7	3-6	6-4	17-2	1-1	10-5	11-4	8-1	2-8	1-6	35-5	80-4
11-7	8-8	6-4	1-1	0-5	0-8	29-2	2-3	0-3	4-2	3-2	5-1	11-8	26-9	10-5	8-7	13-9	2-5	3-5	4-5	43-6	99-6

(although not outnumbering the others). This general result is due to the fact already explained that in 1934 the temperatures were highest at no moon and in 1933 at full moon ; while in 1935 August was one of the months that had a distinct temperature difference in favour of full moon.

However, in spite of this there are distinct differences in behaviour between the groups, and these are summarized in Table XI which shows the groups with their

TABLE XI

Principal Groups of Insects Dealt with Arranged in Sequence of the Difference Between Captures in Full and No Moon weeks

	Mean log diff. per week	Standard deviation	<i>t</i>	Significance
1 Noctuidae (L)	2.77	± 0.44	6.30	certain
2 All insects	2.11	0.94	2.24	probable
3 All Lepidoptera	1.97	0.64	3.01	certain
4 Psychodidae (D)	1.62	1.33	1.22	not sig.
5 Ceratopogon (D)	1.47	0.88	1.67	doubtful
6 Geometridae (L)	1.20	0.61	1.98	possible
7 Mycetophilidae (D)	1.07	0.95	1.14	not sig.
8 Borboridae (D)	0.95	0.88	1.08	" "
9 Crambidae (L)	0.93	0.61	1.52	doubtful
10 Cecidomyidae (D)	0.92	0.89	1.03	not sig.
11 Capidae (R)	0.73	0.64	1.15	" "
12 Chironomidae (D)	0.43	0.94	0.46	" "
13 Coleoptera	0.24	0.40	0.60	" "
14 Psocoptera	0.08	0.68	0.01	" "
15 Jassidae (R)	- 0.24	0.98	- 0.24	" "
16 Aphidae (R)	- 0.85	0.61	- 1.39	" "

L = Lepidoptera. D = Diptera. R = Rhynchota.

mean weekly log difference ; the standard deviation of that difference ; and the " *t* " test of significance (otherwise the mean difference divided by the standard deviation). The groups are placed in order of the value of the log difference.

The following facts may be noted :

(a) The Noctuidae are far above the other groups with a higher log difference, and a very much higher value for *t*.

(b) Out of the first six groups four are either Lepidoptera or include Lepidoptera (*e.g.*, all insects).

(c) Families of Diptera hold positions 4, 5, 7, 8, 10, and 12.

(d) Five out of the last six positions are groups that are neither Lepidoptera nor Diptera.

It would appear from this that the effect was at its maximum in the Lepidoptera, next in Diptera, and lower in other groups. Since all the differences dealt with are logarithmic or geometric ratios the fact that the Diptera make up a large proportion of the catch should not affect their position.

There is, however, one other point of possible importance. Work on the distribution of insects during the night (WILLIAMS, 1935) has shown that Noctuidae have their maximum flight round about midnight (in periods 4 and 5), the Geometridae a little earlier in period 3, and the Crambidae still earlier in period 2. On the other hand, the Chironomidae have their maximum flight at dusk in period 1 with a sub-maximum at dawn (period 8); the Coleoptera and Psocoptera both have their maximum in period 1; the Jassidae resemble the Coleoptera, and the Aphidae had a rather indefinite maximum flight in period 1 in one year and period 2 in the next.

Thus it appears that a possible explanation of the order of sequence in Table XI might be that the insects which fly late are most affected, while those that fly at dusk or dawn (12, 13, 14, 15, 16) are least affected.

In order to test this it is necessary to make a new comparison between full and no moon weeks of the insects caught in each successive period of the night separately.

Effect on all Insects at Different Periods of the Night

Table XII shows for comparison with Table XI the mean differences, standard deviation, and value of t for the 18 full and no moon weeks, for all the insects captured in each of the eight periods of the night separately. It will be seen that: (a) the standard deviation is (with one exception) remarkably constant; (b) the mean difference is always in favour of no moon—it is low in periods 1 and 8 but higher in all the others; (c) the test of significance (t) shows that the results in periods 2–7 are quite significant and approximately equally so.

The probability of the results being due to chance is about 1 : 50. But in period 8 and particularly in period 1, the results although positive are not significant and in the latter period the probability of the result being due to chance is 0.4 : 1.0.

TABLE XII

Difference Between Captures in Full and No moon Weeks, for all Insects, Considered According to the Time of Entry into the Trap

Period	Mean log difference	Standard deviation	t	
1	0.71	0.85	0.83	not significant
2	1.94	0.84	2.30	significant
3	1.82	0.80	2.30	"
4	2.10	0.81	2.60	"
5	2.22	0.87	2.58	"
6	1.81	0.72	2.52	"
7	2.12	0.84	2.50	"
8	1.38	0.85	1.60	doubtfully significant

The lunar influence is therefore very low in the first period at dusk, significant and almost equally high in periods 2–7, and low again at dawn in period 8.

Further, the mean difference in the Noctuidae differs very definitely from the mean values of other insects caught in the same periods (4 and 5), so that the lunar effect on this group at least cannot be entirely due to their time of flight.

On the other hand, the low position of the dawn and dusk flyers, the Coleoptera, Chironomidae, Psocoptera, Jassidae, and Aphidae in Table XI is undoubtedly due in part to the low lunar influence at the times of their flight.

Separate Effect of Moonlight and Cloud on All Insects

Table XIII shows the results obtained when the values for all insects captured each night in all three years together are treated similarly to those for the Noctuidae in Table IX.

The logarithmic mean catch per night was 208 insects. Reducing the values to percentages it will be seen from Table XIII C that on nights of "full moon, no cloud" the catch is 63; on "full moon with cloud" the catch is 115; on "no moon, no cloud" the catch is 81; while on "no moon, cloudy" the catch is 381. All the values in the table are consistent and show quite definitely the separate influence of moon and cloud; but the most remarkable difference from the Noctuid table is the very high value obtained on the nights of no moon with cloud, which is almost three times the value of any of the adjoining groups and nearly six times the number of the opposite extreme "full moon, no cloud".

TABLE XIII

Effect of Various Combinations of Moon and Cloud Conditions on All Insects, for Comparison with Table IX

A. Mean log				B. Mean catch (anti-log - 1)				C. % of mean catch			
2.12	2.16	2.38	2.19	131	144	239	154	63	69	115	74
2.17	2.34	2.43	2.31	147	218	268	203	70	105	129	97
2.23	2.46	2.90	2.50	169	287	793	315	81	138	381	151
2.19	2.32	2.54		154	208	346		74	100	166	

Another curious fact is the small difference between the values for full moon and no moon on nights when there is no cloud.

If one were to attempt an explanation of the differences at the present early stage in the investigation it might be suggested that the insects as a whole (which include a very large majority of small Diptera) are more sensitive to "optimum" conditions, which are cloudy (and hence warm and damp) nights with no moon, and do not fly if these conditions are not existing, while the Noctuidae are more tolerant of their climatic environment and are only seriously reduced by the poorest conditions, which are cool clear nights with full moon.

Effect of Moonlight and Cloud on the Sub-family Tipulinae (Diptera)

PINCHIN and ANDERSON (1936) have applied similar methods to the study of the Tipulinae captured in the trap and have obtained the following values (expressed

as percentages) for the final moon-cloud diagram for the years 1933 and 1934 combined. The results are once more consistent and show a ration of about 4 : 1 between the extremes of "full moon, no cloud" and "no moon, cloudy".

TABLE XIV—EFFECT OF VARIOUS COMBINATIONS OF MOON AND CLOUD CONDITIONS ON THE FAMILY TIPULINAE

54	81	162	77
73	127	162	116
100	139	200	135
73	116	173	

GENERAL DISCUSSION

It would appear from the above results that when certain groups of insects are attracted to a trap by means of a light, the captures show a lunar periodicity with a minimum at full moon and a maximum at or just after no moon. This is particularly definite in the family Noctuidae of the Lepidoptera which have their maximum activity round midnight, but is very much less marked in certain other groups, such as Coleoptera and Jassidae which fly chiefly at dusk and dawn. The Noctuidae, however, differ significantly from other insects flying at the same time.

Certain asymmetries in the effect can be traced to similar asymmetries in the apparent movements of the moon, and when the asymmetry of the moon changes that of the captures does also.

When the cloud effect is analysed separately it is found that cloudy nights have larger captures than clear nights, but these nights are, on an average, distinctly warmer than the clear nights, and this may be sufficient to account for the differences in catches.

The fullest effect of the moon is noticeable on clear nights when the ratio of captures in the Noctuidae between no moon and full moon is 128 : 42 or 3 : 1 ; on cloudy nights the ratio is 184 : 95 or 2 : 1 ; while on all nights irrespective of cloud conditions the ratio is 146 : 54 or about 2.7 : 1.

The reality of the reduction of catches by the moon may therefore be considered to be definitely established.

This reduction may be due to one of two effects. First may be that the moonlight is reducing the activity of the insects, so that the active population available to be sampled by the trap is smaller. Secondly, it is possible that the light of the trap has to compete with the light of the moon and so is less efficient and attracts insects from a smaller area.

It is not at present possible to distinguish between these two alternatives with certainty, but the fact that the Noctuidae differ considerably in their response from other insects flying at the same time suggests the effect may be partly at least physiological.

Further investigation is proceeding to settle this point by the use of traps not dependent on light for their attractive power. During 1935 a mechanical trap was constructed and tested (WILLIAMS and MILNE, 1935), but while very successful for the smaller insects it did not catch the larger Lepidoptera in any numbers. During 1936 experiments are being started on the use of a bait trap.

If a reduction at full moon is shown by these traps, then the effect of the moon must be a general lowering of activity of the insects concerned, as is the popular belief among insect collectors. If on the contrary the mechanical and bait traps show no lunar periodicity, the effect of the moon must be merely to lower the efficiency of the light as an attractant.

SUMMARY

The object of the investigation was to test the truth of a general belief that insect night activity in certain groups, particularly Lepidoptera, is reduced at full moon. This belief was held to apply to insect activity in general, but in particular to the number of insects attracted to light.

A light trap was placed in a field at Rothamsted in March, 1933, and has been in continuous use since that date. The captures of the Noctuidae during the summers of 1933, 1934, and 1935 were selected for special study.

Three main methods of analysis were used. (I) A comparison of total captures and of the sum of the logarithms of captures, in the full moon and no moon weeks of eighteen lunar months, six in each summer. (II) Averaging over the eighteen lunar months the mean departures of the log of the catch from the 29-day mean for each of the 29 days of the lunar cycle. (III) A calculation of the mean departures of the log from the 29-day mean for all days of the period grouped into nine divisions according to moon conditions (full, intermediate, and no moon), and to cloud conditions (clear, intermediate, and cloudy sky).

The amount of night cloud was measured chiefly by an adaptation of the Greenwich pole-star camera; while the moonlight was measured by a specially designed photographic recorder.

In the Noctuidae all three methods gave a definite indication of lunar periodicity. The first comparison showed 17 of the 18 lunar months with a higher catch at no moon than at full moon and a mean difference of six times the standard deviation.

The second method gave a curve with a definite minimum very close to full moon and a maximum slightly after no moon. This curve is asymmetrical and further analysis showed that it was compounded of two curves with opposite asymmetries, each explicable by similar asymmetries in the moon's apparent movements.

The third method shows a ratio of 3 : 1 between no moon and full moon week captures when the sky is clear, and 2 : 1 when the sky is cloudy. The same method shows that the ratio of captures on cloudy nights to clear nights is about 1.75 : 1, but this is associated with warmer conditions on cloudy nights (about 4° F. higher on minimum temperature) due to the reduction of radiation by the clouds.

A difference has been demonstrated between the hours of flight during the night of the Noctuidae in the week before and after full moon, corresponding to the effect of the early or late portion of the night being under the moonlight.

Other groups of insects analysed by the first method show varying lunar effects, never, however, so striking as that in the Noctuidae and in a few groups (*e.g.*, Coleoptera, Jassidae, and Aphidae) it is non-significant or slightly negative.

The groups showing least effect are shown to be those that fly chiefly at dusk or dawn, whereas the Noctuidae have their maximum activity at midnight; but a separate analysis of all insects, according to the different times of the night at which they are captured, shows that this time of flight is not sufficient to account entirely for the difference found.

When the total insects captured are analysed by the third method consistent results are obtained, but with a very large increase in captures on the days of no moon—full cloud. It is suggested that the explanation of this may be a narrowly limited range of optimum conditions of moon, cloud, temperature, and humidity in the smaller Diptera which make up most of the catch.

It is considered that the lunar effect on the captures is definitely demonstrated, and that there is distinct evidence that it differs in different groups apart from any difference in their time of flight. Therefore, it is probably a physiological effect on the activity of the insects and not merely due to reduction in the efficiency of the light trap when the moon is shining. Further experiments to test this more fully are being carried out, by using traps not dependent on light for their attracting power.

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X—Studies on the Embryology of the African Migratory Locust, *Locusta migratoria migratorioides* R. and F.

I—The Early Development, with a New Theory of Multi-phased Gastrulation Among Insects

By MITHAN LAL ROONWAL, *M.Sc., Ph.D. (Cantab.)*

(*From the Entomology Department, Zoological Laboratory, Cambridge*)

(Communicated by A. D. IMMS, F.R.S.—Received October 28, 1935)

[PLATES 33–35]

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I—INTRODUCTION

Notwithstanding the large amount of work done on insect embryology, certain outstanding problems are still productive of much divergence of opinion. The process of gastrulation, the origin of the mid-gut epithelium, and, in short, the general problem as to how far the germ-layer theory is applicable to insects, are matters of dispute to-day. Further, the claim of WIESMANN (1926) to have found the labral

and the preantennary coelom sacs in *Carausius (Dixippus) morosus* (Orthoptera, Phasmidae) has lent a new interest to the problem of head segmentation among Arthropods in general and insects in particular.

The Orthoptera, being the most generalized group among the Pterygota, are specially suited for the investigation of these problems. The family Acrididae was chosen because its embryology has been but little worked out and also because of the ease with which the material for work was available. Among older literature only PACKARD's (1878-83) and GRABER's (1888-91) works deal with members of this family, viz., with *Melanoplus spretus* and *Stenobothrus variabilis* respectively. During recent years, however, owing to the interest created by the locust problem, a number of papers has appeared which deal with the various aspects of the embryology of this family. These papers are by McNABB (1928), SLIFER (1932-4), NELSEN (1931-4), and ELSE (1934). In the present series of papers it is proposed to give as complete an account as possible of the embryology of the African Migratory Locust, *Locusta migratoria migratorioides* R. and F., which is referred to, for brevity, as *Locusta migratoria*.

II—MATERIAL AND METHODS

The eggs used in this work were laid by *Locusta migratoria* L., sub-species *migratorioides* (REICHE and FAIRMAIRE), which is kept breeding from generation to generation in the Entomological Field Station at Cambridge. The original stock consisted of eggs laid by this locust in the *phasis gregaria* near Khartoum. The supply was sent by Mr. A. H. WOOD of the Gezira Agricultural Research Service to Dr. A. D. IMMS who received them in June, 1933. They were transmitted by air mail and arrived in good condition. For the purpose of the present study, only eggs laid by locusts in the *phasis gregaria* were used. In order to obtain accurately timed stages, mating couples were transferred from breeding cages to an incubator maintained at a constant temperature of $33 \pm 0.5^\circ \text{C}$ and the eggs were kept under these conditions throughout the incubation period. The age of the eggs described in this paper refers to the above temperature. The sand containing the developing eggs was always kept moist by adding water to it from time to time.

The dissecting out of the early embryos from the egg was done in a mixture of equal parts of Bouin's fluid and Ringer's solution and fixation was done in Bouin's fluid. For staining embryos in whole eggs thionin gave good results while borax carmine was useless. A variety of fixatives was tried for the eggs. Of these, alcoholic Bouin for early stages and this and Carnoy's acetic alcohol (Formula No. 2) for late stages gave the best results. In order to ensure proper fixation, it was found, necessary to pierce the eggs in several places with a fine needle after placing them in the fixative.

The difficulty of sectioning the yolky locust eggs is accentuated by the presence of a thick and almost impermeable vitelline membrane. Several different methods of sectioning were tried, including the use of soft wax of M.P. 42°C before passing into

harder wax ; double embedding (collodion and wax) ; clearing in aniline oil or in methyl salicylate after 90% alcohol ; clearing in oil of turpentine after 95% alcohol ; and finally, HEIDER's method (1889) of painting with collodion. Of these the last alone gave reasonably satisfactory results, but was very slow. The process finally employed, however, was a modification of PETRUNKEWITSCH's (1933) cupric-phenol fixing method introduced by SLIFER and KING (1933) and improved by ROONWAL (1935). Sections were cut from 5 to 12 μ thick. To prevent thick sections from falling off the slide, the latter, with the sections *in situ*, was dipped into HESSE's (1901) photoxylin solution ($\frac{1}{4}$ to $\frac{1}{2}$ % photoxylin in absolute alcohol and ether) before passing into 90% alcohol while descending from absolute alcohol. For staining sections, the best results were obtained with Heidenhain's iron haematoxylin and orange G.

III—EMBRYONIC DEVELOPMENT

1—Time-table of Early Development

The total duration of incubation at 33° C from the time of egg-laying up to the emergence of the nymph is about 12½–13 days. Blastokinesis occurs at the 5½–6 days' stage. In Table I is given a detailed time-table of the early development :—

TABLE I—TABLE OF EARLY DEVELOPMENT

Age of egg (in hours after egg-laying)	State of development
0–5½	Maturation and fertilization probably occur within 1–2 hours after egg-laying. First and second cleavage divisions.
5½	Stage with 4 cells.*
7	Stage with 6 cells.*
8	Stage with 16–18 cells.*
10	Stage with about 41–45 cells.*
13	The cells are nearing the egg-periphery and have reached half-way up the egg. (Approximately 224 cells present.)*
18	The cells have reached the egg-periphery and form small groups. (Approximately 872 cells present.)*
21	The cells form larger groups than before, especially at the postero-ventral end of the egg. (Approximately 1431 cells present.)*
23	The primary epithelium is formed at the postero-ventral end of the egg. The cells have reached the anterior end of the egg.
28	Completion of the primary epithelium all round the egg with clear differentiation into embryonic and extra-embryonic regions.
30	Cells in the germ disk region divide rapidly, resulting in the temporary loss of its uni-layered condition. First ventral groove and yolk-cell membrane make their appearance.
34	First ventral groove and yolk-cell membrane have disappeared by now. Beginning of degeneration of other yolk cell nuclei also. Many-layered condition of the germ disk persists.

* These figures are based upon actual counts made on individual eggs.

TABLE I—continued

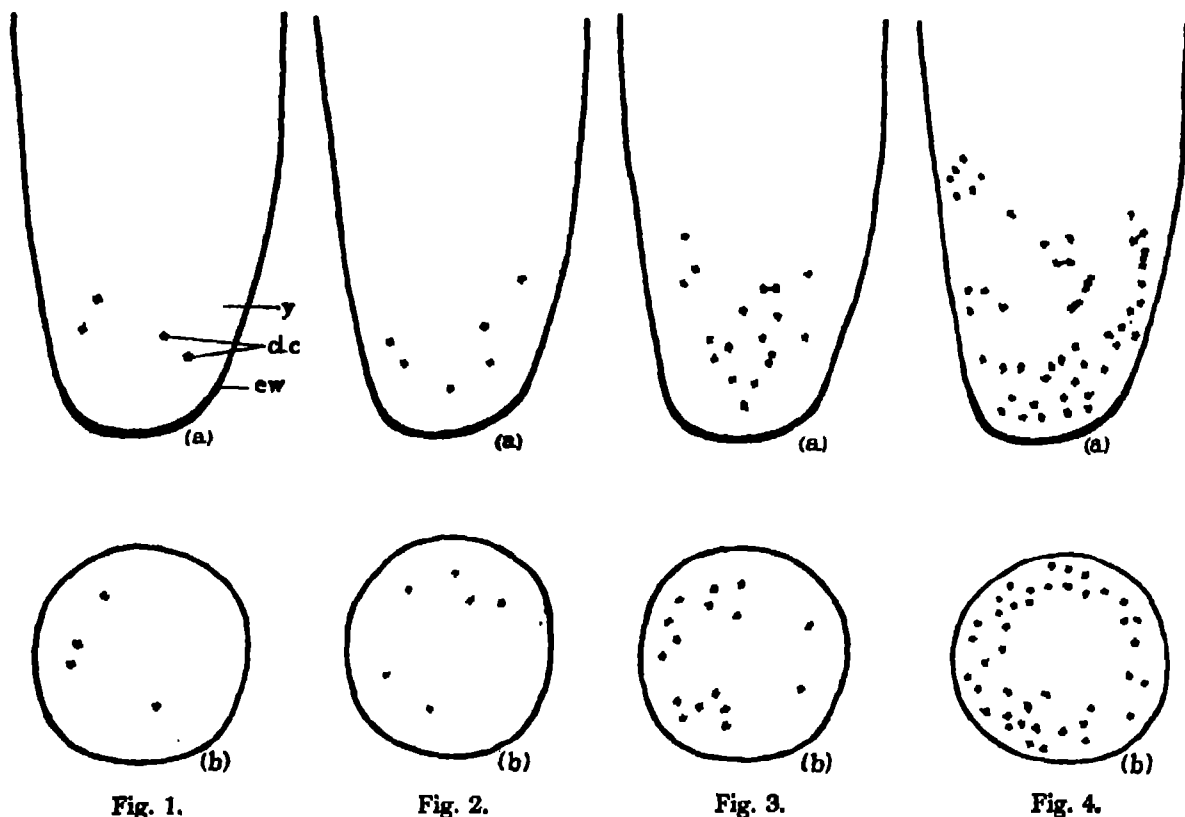
Age of egg (in hours after egg-laying)	State of development
42	Germ band differentiated into protocephalon and protocorm. Beginning of the differentiation of the inner layer (from the roof of the second ventral groove) at the cephalic end of the embryo. Formation of the cephalic fold of the embryonic membranes.
46	Region of inner layer differentiation has extended to the caudal extremity of the embryo. Appearance of the caudal and lateral folds of the embryonic membranes.
50	Completion of the embryonic membranes <i>i.e.</i> , closure of the amniotic cavity. Considerable elongation of the protocorm. Formation of head lobes. Beginning of primary segmentation of the inner layer. Stomodaeal rudiment appears.
Slightly older than 50	Primary external segmentation of the embryo into four segments and a corresponding segmentation of the inner layer. Appearance of antennary rudiments.
52	Paired rudiments of the labrum, jaw and thoracic appendages appear. Stomodaeal invagination formed.
53	Definitive external segmentation of the entire body except the abdomen. Rudiments of the first abdominal appendages appear.
56	Coelomic cavities of the head and thorax complete. Suboesophageal body differentiated.
59	Embryo is very long and thin. Provisional dorsal closure formed and thus epineural sinus arises. Proctodaeum formed. Neuroblasts of the brain differentiated. Optic lobes begin to be delaminated.
64	Neuroblasts of ventral nerve chain differentiated.
75	Definitive external and internal segmentation of abdomen complete. Coelom formation (except that of intercalary segment) complete.

2—Cleavage and the Formation of the Primary Epithelium*

In the present paper the process of fertilization is not dealt with. McNABB (1928) has investigated maturation and fertilization stages in the Acridids *Chrotophaga viridifasciata* and *Circotettix verruculatus*. Recently, SLIFER and KING (1934) have studied the maturation divisions in fertilized eggs of *Melanoplus differentialis*, and KING and SLIFER (1934) in unfertilized eggs of the same insect. It is probable that these processes in *Locusta migratoria* are similar to those described in the above-mentioned Acridids. The earliest cleavage stage studied is the 4-cell stage (fig. 1a, b) which occurs at about 5½ hours after egg-laying. These cleavage cells consist of large, stellate, protoplasmic masses with well-defined central nuclei. They measure, exclusive of the protoplasmic processes, about 16 μ in diameter. So far as could be determined, these cleavage cells are not connected with one another by protoplasmic strands. They cannot, therefore, be said to form a syncytium but are definitive cells. This is in contrast to some other insects where

* The term "primary epithelium" is equivalent to the blastoderm of most authors, the epiblast of GRABER (1891, a) and the Oberflächenepithel of HIRSCHLER (1924). The reasons for its adoption are discussed on p. 409.

up to the 8-nucleate stage definitive cleavage cells are not recognizable. Indeed, in some insects, it is the cleavage nuclei (not cells) which migrate to the egg-periphery, where the periplasm of the egg provides the cytoplasm round them. All the four cleavage cells lie very close to the posterior end of the egg, the furthestmost cell being only about $495\ \mu$ from the posterior pole. The cells lie distinctly away from the periphery.



FIGS. 1-4—Posterior regions of eggs, showing various stages of early cleavage. The figures have been drawn as sections with all the cells projected in a single plane. \times about 27. (a) Longitudinal sections of posterior regions of eggs. (b) Transverse sections near posterior poles of eggs. *cl. c.*, cleavage cells; *ew.*, egg-wall; *y.*, yolk.

FIG. 1—5½ hours old. 4 cleavage cells.

FIG. 2—7 hours old. 6 cleavage cells.

FIG. 3—8 hours old. 16 cleavage cells.

FIG. 4—10½ hours old. 45 cleavage cells in (a) and 41 in (b).

At about the 7 hour stage (fig. 2*a, b*) six cells are seen. They have begun to migrate anteriorly, a process which becomes more and more pronounced as cleavage progresses. The farthest cell at this stage is about $560\ \mu$ from the posterior pole.

At the 8 hour stage (fig. 3*a, b*) already 16 cells are present, two of which are seen dividing (fig. 3*a*), being probably in the late telophase stage. The farthest cleavage

cell is about $760\ \mu$ from the posterior pole. The division rate, which ~~was~~ rather slow until the 7 hour stage, has now rapidly increased.

At the $10\frac{1}{2}$ hour stage (fig. 4a, b) about 41–45 cells could be counted, the deepest cell being about $1066\ \mu$ from the posterior pole of the egg. The cells have now started to migrate towards the egg-periphery. The disposition of the division spindles in these early stages do not show any definite orientation to the egg-periphery, a fact which is in accord with what has been described in the majority of insects so far studied.

All the cleavage cells are stellate but none of them shows comet-like protoplasmic extensions streaming behind the migrating cells (*cf.* EASTHAM, 1927 ; SEHL, 1931 ; and others). In the present case, therefore, the migration of the cleavage cells to the egg-periphery should be described not as a nuclear movement with the dragging of the cytoplasm behind the nucleus, but as a movement of the entire cell. In other words, the impetus of migration is shared equally by the nucleus and the cytoplasm. The cause of the migration has been ascribed by several workers to amoeboid movement. In the locust eggs, however, there is no evidence of such a movement, and PATTEN's (1884) idea, also accepted by EASTHAM (1927), of some centrifugal influence propelling the cells towards the periphery seems more acceptable.

At the 13 hour stage (fig. 5a and fig. 17, Plate 33) the cells are close to the egg-periphery. Their nuclei are rounded and the cytoplasm no longer shows stellate processes except in the cells left behind as primary yolk cells. The extremely fine periplasm can be seen only with difficulty. Vertically the cells have reached about half-way up the egg. From now onwards this vertical migration occurs along the egg-periphery and not through the yolk (fig. 7).

By the 18 hour stage (figs. 18 and 19, Plate 33) the cells have reached the egg periphery where they flatten out temporarily. This flattening of the cells immediately on reaching the egg-periphery is very probably a surface tension phenomenon. Such cells consist of a flattened nucleus with a thin coating of cytoplasm. They also show a tendency towards grouping. The yolk in the immediate neighbourhood of these cells is seen to be divided into very fine particles which are probably in a phase of digestion.

By the 21 hour stage (fig. 5b and fig. 20, Plate 33) the cells have migrated still further anteriorly but have not yet reached the anterior pole. The tendency towards grouping of cells, evident in the 18 hour stage, is here still more marked, especially at the postero-ventral end (fig. 39, Plate 35).

At about the 23 hour stage (fig. 5c and figs. 21 and 22, Plate 33) the cells at the posterior end of the egg are in a continuous layer, the primary epithelium, which forms the cup-shaped germ disk where the nuclei are arranged more or less regularly without forming any special groupings (*cf.* fig. 40, Plate 35). The cells on the rest of the egg-periphery do not as yet form a continuous layer. Those cells of the primary epithelium which form the germ disk are somewhat spindle-shaped, being elongated along the egg-periphery. Their nuclei are elongate-oval. The other peripheral cells are more rounded and so are their nuclei (fig. 23, Plate 33).

The mode of division of the primary epithelial cells is mitotic. The division spindles are tangential to the surface of the egg and consequently cell division occurs at right angles to the periphery.

At about the 28 hour stage (figs. 25 and 26*a, b*, Plate 33) the primary epithelium forms a continuous layer all over the surface of the egg. The cells of the embryonic or germ disk region of the primary epithelium are closely packed together. They are columnar, being elongated perpendicularly to the egg-periphery, and have rounded nuclei. On the other hand, the cells of the extra-embryonic region of the

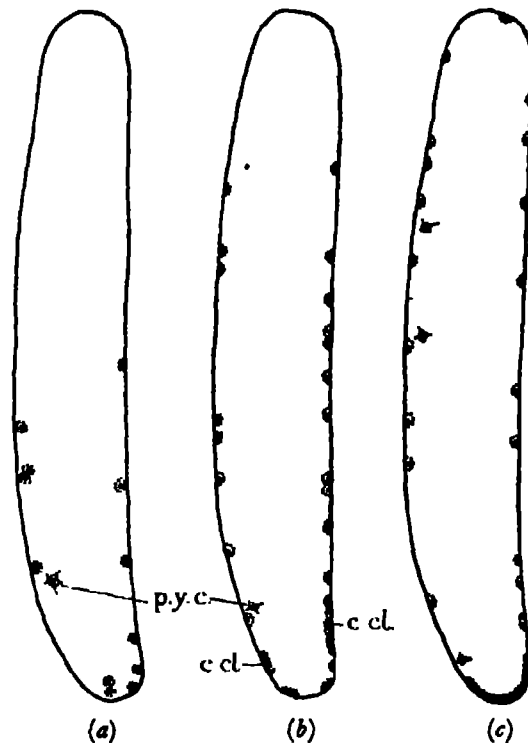


FIG. 5—Longitudinal sections of eggs, showing the migration of cleavage cells. Semi-diagrammatic. \times about 16. (a) Egg 13 hours old; (b) egg 21 hours old; (c) egg 23 hours old. *c.cl.*, cell clusters; *p.y.c.*, primary yolk cells.

primary epithelium, as well as their nuclei, are elongated tangentially to the egg-periphery. At the junction of the two areas, cells of one kind gradually merge into those of the other.

Discussion on the Formation of the Primary Epithelium—As will be seen from the foregoing account, the formation of the primary epithelium in *Locusta migratoria* does not occur simultaneously all over the egg-periphery. At first the posterior half of the egg is reached by the migrating cells. Then, by migration along the egg-periphery, the anterior half is covered. The definitive primary epithelium which forms the germ band first appears at the postero-ventral end of the egg and from there this epithelium formation spreads all over the egg. In other Orthoptera and Dermaptera

the first appearance of the primary epithelium is sometimes simultaneous (*Forficula*, HEYMONS, 1895) sometimes localized. Thus in *Gryllus* and *Periplaneta* (HEYMONS, 1895) it first appears at the hinder pole of the egg and then proceeds forwards. In *Gryllotalpa* (KOROTNEFF, 1885; HEYMONS, 1895; NUSBAUM and FULINSKI, 1909) it first appears on the ventral surface, leaving the dorsal surface uncovered. In *Carausius morosus* (LEUZINGER, 1925) a somewhat peculiar condition obtains. The primary epithelium first appears at the hinder pole of the egg, but the cleavage cells reach the neighbourhood of the anterior end only very late, viz., when the germ disk has already differentiated into the protocephalon and the protocorm; further, these latter cleavage cells are very sluggish and do not divide. The anterior egg-pole itself remains entirely devoid of cells. Such a localized appearance of the primary epithelium has been noted in various insects, for instance, in *Hydrophilus* (HEIDER, 1889), *Donacia* (HIRSCHLER, 1909), *Chalicodoma* (CARRIÈRE and BÜRGER, 1897), *Pieris* (EASTHAM, 1927), *Ephastia* (SEHL, 1931) and in many others. According to

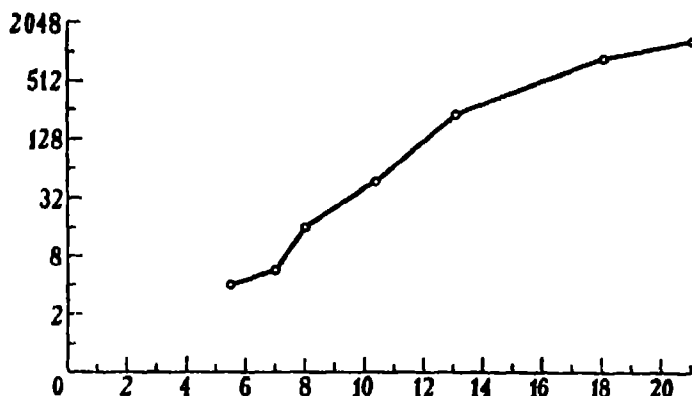


FIG. 6—Graphic representation of the time relations of early cleavage stages until the 21 hour stage.

HIRSCHLER (1924), in spherical or slightly oval eggs, as for example those of *Forficula* and *Campodea*, the primary epithelium appears simultaneously on the entire surface of the egg. In elongated eggs it develops at different places of the egg surface at different times. This classification, however, cannot be regarded as a rigid one because in *Carausius* (LEUZINGER, 1925), for instance, the egg is spherical, yet the primary epithelium appears first at the hinder pole.

Division and Migration Rate of Cleavage Cells—The approximate number of cleavage cells (including vitellophages) and of vitellophages recorded during early development is shown in Table II.

TABLE II
CLEAVAGE CELLS (INCLUDING VITELLOPHAGES)

Hours	5½	7	8	10½	13	18	21
Number	4	6	16-18	41-45	224	872	1431

VITELLOPHAGES

Hours	13	18	21
Number	47	45	36

In fig. 6, these results are expressed graphically. It is seen that the division is most rapid during the 7 to 10 hour period, after which there is a progressive slowing down. The period of greatest division activity thus coincides with a period during which the cleavage cells manifest no other activity. As soon as the cells begin to migrate towards the egg-periphery (at about the 10½ hour stage), the division rate slows down. This conclusion agrees with similar results obtained by SEIDEL (1929) in the Libellulid *Platynemis pennipes* and by SEHL (1931) in the moth *Ephestia kuehniella* ZELL.

As already mentioned, the migration of the cleavage cells towards the egg-periphery is first evident at about the 10½ hour stage. After about the 13 hour stage, all the cells except the vitellophages have nearly reached the egg-periphery. Since both the distance travelled by these cells and the period during which this is accomplished are short, it is not possible to measure the rate of this migration. However, regarding vertical migration, *i.e.*, towards the anterior pole of the egg, the measurement of the migration rate is possible. Even during early cleavage stages when all the cells are more or less clustered together at the posterior pole of the egg, forming a sort of cylinder (fig. 4b), short but definite migration forward occurs. For the sake of convenience, this migration has been measured as the distance of the farthest cleavage cells from the posterior pole of the egg. From about the 13 hour stage onwards, this vertical migration is accomplished along the egg-periphery (fig. 7). At the 13 hour stage nearly half the length has been traversed and by about the 23 hour stage the anterior end of the egg is reached. During early development the approximate distance of the farthest cleavage cell from the posterior pole of the egg is shown in Table III.

TABLE III

Hours . . .	5½	7	8	10½	13	21	22½
Distance in μ	495	580	760	1068	2720	4400	5600

It is interesting to compare the migration rate of the cleavage cells with their division rate. At about the 10½ hour stage when the rate of migration rapidly rises, the division rate rapidly falls. SEIDEL (1932) showed that in *Platynemis* even the early cleavage nuclei undergo considerable migration, but in a definite manner. In *Locusta migratoria* such migration evidently does not occur. SEHL (1931) found that in *Ephestia kuehniella* the cells remain in a mass in the anterior third of the egg until the 32-cell stage. Afterwards they migrate towards the egg-centre, reaching the egg-periphery in about the 512-cell stage.

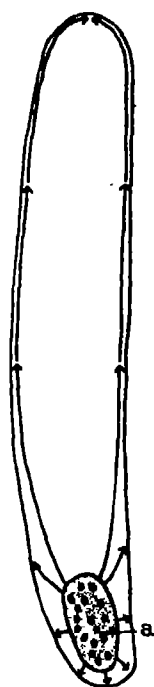


FIG. 7—Diagrammatic representation of the course of migration of cleavage cells after the 8 hour stage. (a) represents the extent of distribution of cleavage cells at the 8 hour stage. The arrows indicate the approximate paths of the cleavage cells.

3—First Ventral Groove and Yolk-Cell Membrane

At about the 30 hour stage (fig. 27, Plate 33) it is seen that the cells of the germ disk epithelium are undergoing rapid division. This results in the epithelium temporarily losing its uni-layered condition and acquiring an irregular two or even three layered disposition of the nuclei at places. Such a condition has also been described in the Phasmid *Carausius morosus* (LEUZINGER, 1925). At this stage there appear two extremely interesting phenomena which are important firstly, because they have been so far described only in one or two other insects; and secondly, because of their considerable theoretical significance in regard to the process of gastrulation and the origin of the primary endoderm among insects. They are described below.

First Ventral Groove (fig. 8 and figs. 27 and 28, Plate 33, and fig. 29, Plate 34)—At the above-mentioned stage there is seen, in the mid-ventral line of the germ disk and lying near the future cephalic end, a shallow, elongated groove on the outer face of the epithelium. It is about 88 μ long, 24 μ wide, and only 6 μ deep. From its roof several rounded cells are seen proliferating. These cells have nothing to do with the formation of the inner layer and very probably form secondary yolk cells. The whole appearance of the groove, combined with the proliferation of rounded cells from its roof, gives it the appearance of the so-called gastral groove. From this, however, it is to be distinguished since the latter makes its appearance some time afterwards, and has been termed in this paper as the "second ventral groove" in contradistinction to the "first ventral groove" described herewith. The first ventral groove lasts for about four hours or less. NELSEN (1934, b), while describing the process of "gastrulation" in the Acridid *Melanoplus*

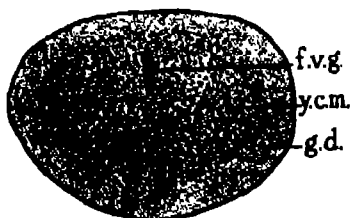


FIG. 8—Diagrammatic representation of the germ disk about 28 hours old, showing the first ventral groove and the yolk-cell membrane. Reconstructed from transverse sections. g.d., germ disk; f.v.g., first ventral groove; y.c.m., yolk cell membrane.

differentialis, makes no mention of the first ventral groove. However, it is evident from his figures (his Plate 1, fig. 1, and Plate 3, figs 17–21) that the condition represented in them corresponds to the first ventral groove (coupled with the many-layered condition of the germ disk which he has confused with inner layer formation).

Such a first ventral groove, as occurs in *Locusta migratoria*, has been recently reported in two other insects, viz., in *Pieris rapae* (Lepidoptera) by EASTHAM (1927) and in *Calandra granaria* (Coleoptera) by INKMANN (1933). In *Pieris* it lies near the cephalic end of the embryo on the ventral side and cells are proliferated from its roof as well as from a mid-ventral line behind the groove. These cells have been shown by EASTHAM to degenerate. The second ventral (gastral) groove of *Pieris* appears afterwards and is continuous with the first one. In *Calandra granaria* this groove (or "Ventralrinne" as INKMANN calls it in contrast to the later appearing "Primitiverinne" or second ventral groove) is short-lived as in *Locusta migratoria* and is not transformed directly into the second ventral groove. In *Gryllotalpa*,

KOROTNEFF (1885) found that although no "gastral" groove is formed, there occurs, soon after the completion of inner layer differentiation, a shallow, ventral, groove-like invagination which, however, bears no relation to the differentiation of the inner layer. So far as I am aware, there are no other references in literature to such "extra-gastral" invaginations. Their theoretical significance is discussed under "gastrulation" (p. 408).

Yolk-Cell Membrane (fig. 8 and fig. 27, Plate 33, and fig. 29, Plate 34)—Simultaneously with the appearance of the first ventral groove it is also seen that the yolk cells lying immediately beneath the germ disk are arranged in a layer. No cell boundaries are visible and the nuclei are connected by protoplasmic strands so as to form a continuous membrane of a syncytial nature. It meets the germ disk some distance inwards from the edge of the latter and extends beneath it as shown in fig. 8. By the 34 hour stage it has degenerated and no trace of it can be seen.

Such a yolk-cell membrane was first described among insects by HAMMERSCHMIDT (1910) in the Phasmid *Carausius* (*Dixippus*). He showed that, like the rest of the yolk cells, it degenerates. LEUZINGER (1925) and LEUZINGER and WIESMANN (1925) confirmed this in the same insect and further showed that this membrane takes some share in the formation of the mid-gut epithelium. These authors regard it as representing the primary endoderm which "fallen, . . . nachdem sie ihre Aufgabe als primäres, vorläufiges Mitteldarmepithel erfüllt haben, der Degeneration, der Auflösung anheim" (LEUZINGER and WIESMANN, 1925, p. 102). Thus, while in *Locusta migratoria* this membrane is extremely short-lived and its degeneration starts simultaneously with the first indication of degeneration of the other yolk cells, in *Carausius* this occurs some considerable time afterwards. The two structures are, however, homologous and represent a part of the primary endoderm. HEYMONS (1901) found such a membrane in *Scolopendra* where, however, it is not restricted to the germ band region but covers the entire yolk. EASTHAM's (1927) "limiting membrane to the yolk" may also belong to this category.

4—Second Ventral Groove and Differentiation of Inner Layer

At about the 34 hour stage (fig. 31, Plate 34) the germ disk is more markedly many-layered than before, although the cells do not exhibit any regular arrangement. Indeed, it would perhaps be more correct to speak of the germ disk as a syncytium. This condition is only temporary and disappears before the differentiation of the inner layer begins, so that this latter process takes place in an uni-layered germ disk. A multi-layered and syncytial early germ disk has been described in several other Orthoptera, viz., in *Carausius* (HAMMERSCHMIDT, 1910; and LEUZINGER, 1925), *Grylotalpa* (HEYMONS, 1895) and in others. In these insects, however, there is no second ventral (gastral) groove and the multi-layered condition is intimately connected with the differentiation of the inner layer. Thus, LEUZINGER says: "Die Bildung der beiden primären Keimblätter erfolgt bei *Carausius* . . . gleichzeitig mit der Blastoderm- und Keimstreifbildung", although "Die Zellen der beiden Blätter können voneinander nicht unterschieden werden".

At about the 42 hour stage (fig. 42, Plate 35) the inner layer (the so-called "unteres Blatt", hypoblast, mesoderm or endo-mesoderm) is seen to arise as a proliferation of cells from the roof of a deep median-ventral groove. This groove, which I have termed the "second ventral groove" (*vide* discussion below), first makes its appearance at the cephalic end of the embryo a short distance from the extreme edge of the germ band. It extends rapidly towards the caudal end, and finally runs almost along the entire length of the embryo with the exception of the extreme cephalic end (figs. 38 and 43, Plate 35). In fig. 33, Plate 34, this groove is shown in section. The cells and nuclei of the inner layer, as well as those ectodermal cells and their nuclei which lie in the immediate neighbourhood of the second ventral groove, are rounded and smaller than the rest of the ectodermal cells and nuclei. The latter are columnar and their nuclei are oval. They are elongated at right angles to the egg-periphery. The second ventral groove is deep and well-marked. It lasts only for about three to four hours and is not met with after about the 46 hour stage. Fig. 38, Plate 35, which is a median-vertical longitudinal section of the posterior end of an egg 46 hours old, shows that the inner layer extends as a continuous layer from the caudal end of the embryo to a little distance behind the extreme cephalic end. It is, however, not of uniform thickness throughout its length, being much thicker at the caudal than at the cephalic extremity (also *cf.* figs. 34-36, Plate 34).

GRABER (1888, *b*, and 1891, *a*) had previously observed a similar mode of inner layer formation in the Acridid *Stenobothrus variabilis* Fieb. More recently, NELSEN (1934, *b*) has described it in *Melanoplus differentialis* although, as pointed out above, he has failed to distinguish between the first and second ventral grooves. His remark that the "inner germ-band layer is formed by invagination, middle-plate formation and cell-proliferation" is apparently not correct. Inner layer formation in the Acrididae, as known from the examples mentioned above, occurs only by means of cell-proliferation from the roof of a mid-ventral, longitudinal invagination, here termed the second ventral groove.

Soon after its origin, the irregular mass of the inner layer (fig. 33, Plate 34) becomes wedge-shaped and fits into a similar notch in the germ band (figs. 34-36, Plate 34). The ectoderm now becomes more than one layered at places and the lateral halves of the germ band are thicker in the middle than at the sides. Shortly after the formation of the second ventral groove, the ectoderm bordering it in the cephalic region appears to be thickened into a pair of short, elongate swellings. These swellings are temporary and soon disappear (fig. 43, Plate 35).

Discussion—The mode of the formation of the inner layer among the Orthoptera is very varied but may be classed under either of these two principal heads, viz., origin by (*a*) invagination and (*b*) immigration. Table IV summarizes our present knowledge of inner layer formation in the Orthoptera and the Dermaptera.

It will be seen from Table IV how varied is the method of inner layer formation even in the closely related genera of the Orthoptera. All the three members of the family Acrididae so far studied, viz., *Stenobothrus variabilis*, *Melanoplus differentialis*,

TABLE IV—INNER LAYER FORMATION IN THE ORTHOPTERA AND THE DERMAPTERA

a. INVAGINATION	b. IMMIGRATION
(proliferation from the roof of a mid-ventral groove—the second ventral groove).	(proliferation occurs either (i) all over the germ band or (ii) mostly from special lateral areas of the germ band ; not from the roof of a groove).
ACRIDIDAE	PHASMIDAE
<i>Stenobothrus variabilis</i> (GRABER, 1888, b, 1891, a). <i>Melanoplus differentialis</i> (NELSEN, 1934, b). <i>Locusta migratoria migratorioides</i> R. and F. (ROONWAL—present paper).	<i>Carausius (Dixippus) morosus</i> (HAMMER-SCHMIDT, 1910 ; STRINDBERG, 1914 ; LEUZINGER, 1925).
BLATTIDAE	BLATTIDAE
<i>Blatella (Phyllodromia) germanica</i> (WHEELER, 1889 ; CHOLODKOWSKY, 1891. But cf. HEYMONS, 1895 ; and NUSBAUM and FULINSKI, 1906). <i>Periplaneta orientalis</i> (HEYMONS, 1895).	<i>Blatella (Phyllodromia) germanica</i> (HEYMONS, 1895 ; NUSBAUM and FULINSKI, 1906. But cf. WHEELER, 1889 ; and CHOLODKOWSKY, 1891).
GRYLLIDAE	GRYLLIDAE
<i>Oecanthus</i> (AYERS, 1884. Groove very shallow). <i>Gryllus</i> (WHEELER, 1893 ; HEYMONS, 1895).	<i>Gryllotalpa</i> (KOROTNEFF, 1885 ; HEYMONS, 1895 ; NUSBAUM and FULINSKI, 1909).
MANTIDAE	FORFICULIDAE
<i>Mantis</i> (GRABER, 1878 ; BRUCE, 1887 ; VIALLANES, 1891). <i>Stagmomantis</i> (WHEELER, 1893).	<i>Forficula</i> (HEYMONS, 1895. Mostly from lateral borders of germ band).
TETTIGONIIDAE	
<i>Xiphidium</i> (WHEELER, 1893).	
and <i>Locusta migratoria migratorioides</i> , possess a deep, ventral, groove-like invagination extending almost throughout the entire length of the embryo and from whose roof the inner layer is proliferated. Of the other families, some show only one, while others show both modes of inner layer formation as shown below :—	
(a). Inner layer formed only through invagination.	(b). Inner layer formed only by diffuse or by localized immigration.
Acrididae. Tettigoniidae. Mantidae.	Phasmidae.
(c). Inner layer formed by both modes, (a) and (b).	
Blattidae. Gryllidae.	

The above grouping is only tentative because, although in some insects the presence or absence of the second ventral groove has been demonstrated beyond doubt, in others the evidence is either conflicting (as, for instance, in *Blatella* (*Phyllodromia*) *germanica*) or is otherwise unsatisfactory, being based on faulty technique, so as to demand re-investigation. In still others the number of representatives studied from a particular family is only either one or two.

In *Locusta migratoria* the differentiation of the inner layer occurs from a single area, viz., from an elongated median-longitudinal line, that is to say, its developmental mode is unitary (to use the term of HIRSCHLER, 1924) and localized. Such a unitary mode of inner layer formation has been described by WHEELER (1889) in *Blatella* (*Phyllodromia*) *germanica* where the inner layer arises as a small area at the hind end of the germ band and progresses forwards. This condition is contrary to that which occurs in *Locusta migratoria*, where the differentiation of this layer proceeds from the cephalic towards the caudal end. On the other hand, in some other Orthoptera the inner layer develops in the beginning as two or more well-marked patches which grow towards each other and unite, thus acquiring a unitary character only secondarily. This occurs, for example, in *Gryllotalpa* (NUSBAUM and FULINSKI, 1909) where the inner layer arises from four separate rudiments. With regard to the area which the inner layer at the time of its formation occupies in relation to the entire extent of the germ band, two extremes can be distinguished among insects. In one the inner layer is restricted to a small area of the germ band, i.e., it is localized. This occurs in *Lepisma* (HEYMONS, 1897, a). In the other, the inner layer extends almost over the entire surface of the primary epithelium, in other words, it is diffuse. This condition obtains in *Isotoma* (PHILIPTSCHENKO, 1912, among the Apterygota; among the Pterygota it occurs in some Orthoptera, viz., *Blatella* (*Phyllodromia*) *germanica* (HEYMONS, 1895), and *Gryllotalpa* (HEYMONS, 1895; NUSBAUM and FULINSKI, 1909), and in the Isopteran genus *Eutermes* (KNOWER, 1900). The majority of the other insects, including *Locusta migratoria*, occupy a position mid-way between these two extremes. It should be pointed out that the presence of a localized, or of a diffuse, mode of inner layer formation does not appear to have any phylogenetic significance. The Apterygota show both the modes and so do the Pterygota.

5—Yolk and Yolk Cells

Yolk—The structure of the yolk has been studied in sections cut by HEIDER's method (1889) of painting with collodion. At the time of oviposition, the yolk of *Locusta migratoria* is like a viscid fluid composed of large and small yolk spheres, with minute droplets of fat scattered throughout the whole egg. The yolk spheres have a characteristic distribution. Except at the poles and the egg-periphery, they are large and more or less rounded. Near the poles, however, they become smaller, giving the yolk a granular appearance. All round the egg-periphery a thin layer of yolk composed of very minute particles can be distinctly seen. The protoplasmic reticulum is not distinct in the early stages, but, when the amount of yolk is diminished

in older stages, it becomes evident. In *Blatella* (*Phyllodromia*) *germanica* (PATTEN, 1884) a similar distribution of yolk occurs, with the difference that the granular yolk is not specially abundant at the poles. The peculiar formation of two zones described in the same insect by BLOCHMANN (1887) does not occur in *Locusta migratoria*. The yolk spheres are at first closely packed together. As the egg grows older, they become sparsely distributed. At the same time, the yolk mass is pushed towards the anterior end of the egg, the posterior end being occupied by the embryo. Also, evidently as a result of assimilation, the yolk changes in character. This is shown by its greater ease and less brittleness in cutting in the older stages than in the younger ones. The yolk is also less viscid than formerly.

Secondary Yolk Cleavage—The most important change that the yolk undergoes, from the morphological point of view, is the so-called secondary yolk cleavage (fig. 9). At about the 60 hour stage the yolk, which has so far remained "amorphous" and taken no share whatever in the original cleavage processes, begins to show polyhedral differentiation in the immediate neighbourhood of the embryo. This feature spreads to the rest of the yolk in a postero-anterior direction. By the 75 hour stage, the entire yolk becomes divided into polyhedral masses, which can be seen externally even in the living egg. This phenomenon lasts for about 24 to 36 hours and then completely disappears so that in an egg about 100 hours old no trace of it can be seen. The yolk polyhedrals are sharply defined and each generally contains a large nucleus, although occasionally enucleate examples are found.

This transient phenomenon of secondary yolk cleavage is of considerable theoretical importance from the point of view of comparative morphology. It has been described in the Orthoptera, the Dermaptera, the Coleoptera, and the Lepidoptera and is termed "secondary" in contradistinction to "primary yolk cleavage" occurring in the majority of the Apterygota and in some parasitic Hymenoptera and the Strepsiptera. The ant *Azteca* (STRINDBERG, 1916) is of special interest since it is the only non-parasitic Pterygotan in which a total cleavage (and thus a primary yolk segmentation) occurs. STRINDBERG (1913-19) has also described in several other ants (*Camponotus*, *Leptothorax*, *Formica*) an early partial cleavage of the superficial ("sub-blastodermal") yolk, resulting in the formation of yolk pyramids—a phenomenon partly comparable to total cleavage. It is interesting to compare these yolk pyramids with those occurring in the other Arthropoda. Thus, they occur in several Myriapods (HEYMONS, 1901, *Scolopendra*; and others), Crustacea (REICHENBACH, 1886, both primary and secondary yolk pyramids; MANTON, 1928, *Hamimysis*, secondary yolk pyramids; and others), Arachnids (MORIN, 1886,

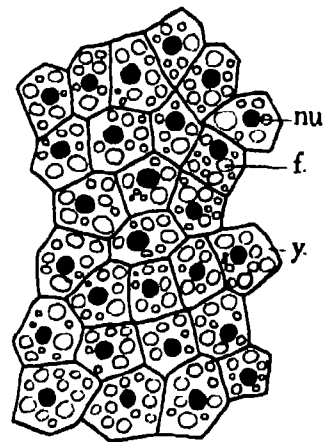


FIG. 9—A portion of the yolk from an egg 70 hours old, showing secondary yolk cleavage (semi-diagrammatic). \times about 75. *f*, negative images of fat globules; *nu*, nucleus; *y*, yolk.

Theridium ; and others), Pantapoda (MORGAN, 1891 ; and others), and finally the Onychophora (SHELDON, 1888, *Peripatus novaezealandiae*). This, and similar evidence, which need not be marshalled here, point towards the fact, as suggested by STRINDBERG, that the secondary yolk cleavage is only a belated expression of true cleavage activity, and the yolk polyhedra are comparable to true yolk cells. With this view I am in complete agreement. What share then do these yolk cells, or, in some cases, yolk syncytia, take in the further development of the embryo ? For the majority of insects the older view of the origin of the mid-gut epithelium from yolk cells is no longer held. But in *Lepisma* and *Campodea* (HEYMONS, 1897, *a, b*), in Libellulids (TSCHUPROFF, 1903), and in a few other insects the definite origin of the mid-gut epithelium from yolk cells has been shown. Of special interest is TSCHUPROFF's observation that in the Libellulidae only the middle part of the mid-gut epithelium is of yolk cell origin, the rest being ectodermal. This suggests the gradual obliteration of the yolk cell element from the mid-gut of higher insects. In the majority of the primitive Pterygota, the yolk cells, after their sudden and belated outburst of activity in the form of secondary yolk cleavage, soon lose all morphogenetic value and degenerate. In some other Pterygota, even this expression of activity has been suppressed.

Yolk Cells—In *Locusta migratoria* not all the cleavage cells reach the egg-periphery to form the primary epithelium. Some remain beneath this layer and form the so-called vitellophages or primary yolk cells. In other words, "intra-vitelline separation" of HEYMONS occurs. The primary yolk cells retain the appearance of early cleavage cells, *i.e.*, they are stellate and have large, round nuclei. During the early stages of intravitelline separation, all the yolk cells are more or less superficial in position. At about the 13 hour stage when the cleavage cells have nearly reached the egg-periphery, the number of primary yolk cells is about 47 out of a total of about 224 cleavage cells. It is possible that some of the former may, at this early stage, still represent tardy cleavage cells which have not reached the egg-periphery. Their number gradually decreases up to the 21 hour stage when there are only about 36 primary yolk cells out of a total of about 1431 cleavage cells. After this stage, however, there is a rapid increase in their number due to the formation of secondary yolk cells. Thus, at about the 23 hour stage, nearly 119 vitellophages could be counted, all lying near the periphery of the egg.

Our present knowledge of the presence or absence of an intravitelline separation among the Orthoptera is summarized in Table V.

The various ways by which the secondary yolk cells of *Locusta migratoria* arise are described below. Firstly, they may arise by migration of primary epithelium cells. Individual cells become amoeboid and migrate centripetally from the egg-periphery (figs. 21 and 24, Plate 33). This mode is multipolar and not restricted to any special region of the egg-periphery. It was first recorded by WHEELER (1889) in *Blattella* and afterwards by HEYMONS (1895) in other Orthoptera and Dermaptera. SCHWANGART (1904 and 1906) classified insects into two groups, *viz.*, those where such a migration is multipolar and those where it is localized, no intermediate

TABLE V—THE ORIGIN OF YOLK CELLS IN THE ORTHOPTERA

Intravitelline separation occurs— (primary yolk cells formed)	No intravitelline separation occurs— (no primary yolk cells formed)
ACRIDIDAE <i>Locusta migratoria migratorioides</i> R. and F. (ROONWAL—present paper).	PHASMIDAE <i>Carausius (Dixippus) morosus</i> (HAMMER- SHMIDT, 1910; LEUZINGER, 1925).
GRYLLIDAE <i>Gryllus</i> (HEYMONS, 1895). <i>Oecanthus</i> (AYERS, 1884). <i>Gryllotalpa</i> (NUSBAUM and FULINSKI, 1909. But cf. KOROTNEFF, 1885, and HEYMONS, 1895).	GRYLLIDAE <i>Gryllotalpa</i> (KOROTNEFF, 1885, and HEY- MONS, 1895. But cf. NUSBAUM and FULINSKI, 1909).
MANTIDAE <i>Mantis</i> (GIARDINA, 1897).	BLATTIDAE <i>Blatella (Phyllodromia) germanica</i> (WHEELER, 1889; HEYMONS, 1895). <i>Periplaneta</i> (WEISMANN, 1882); KOROTNEFF, 1885; HEYMONS, 1895).

It will be seen that among the Gryllidae both the modes occur.

condition occurring. This classification, however, is not acceptable because both modes may occur in one and the same insect, as for example, in *Calliphora* (NOACK, 1901). A second mode is by the division of the primary epithelium cells. Certain cells become amoeboid and divide. The inner product of such a division is interpreted as forming a secondary yolk cell (fig. 10a, b). This mode has not been described previously in any other insect. The origin of secondary yolk cells from primary yolk cells could not be ascertained as no division stages of the latter have been met with.

The secondary yolk cells were observed undergoing division in very few instances only, and in these examples no mitotic stages could be detected. It is, therefore, not possible to state definitely which type of nuclear division (mitosis or amitosis) prevails. In other insects, however, these cells have been shown to divide both amitotically (SCHWARTZE, 1899; TOYAMA, 1902; MARSHALL and DRENHEHL, 1906; and others) and mitotically (NELSON, 1915; HUTE, 1917; EASTHAM, 1927; SEHL, 1931; and others).

In about the 28 hour stage the yolk cells immediately beneath the germ disk arrange themselves in a single layer. This layer, at about the 30 hour stage, is seen to form the yolk-cell membrane described above. Of the other yolk-cells which do not share in the formation of this membrane, some lie singly and are stellate, while others clump together into twos and threes or more to form irregular syncytia which do not

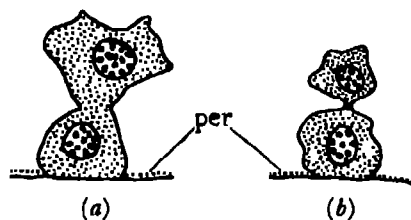


FIG. 10—Two late stages in the formation of a secondary yolk cell by division of a primary epithelium cell. From the posterior pole of an egg about 23 hours old. $\times 370$. a, Early stage; b, stage near completion of division. *per.*, periplasm.

show any stellate processes (figs. 29 and 30, Plate 34). These yolk-cell syncytia become enlarged probably by amitotic division of their constituent nuclei. At the 34 hour stage, no trace of the yolk-cell membrane is seen—it has undergone complete degeneration. At this stage, also, some of the other nuclei show the onset of degenerative changes (fig. 31, Plate 34). This degeneration of yolk cells is, however, considerably slower than their reinforcement, so that their number continuously increases for a long time. The degenerative changes in a yolk cell occur in a manner similar to that described by WIESMANN (1926) in *Carausius*. At about the 40 hour stage the yolk-cell syncytia described above are seen to be still more enlarged and may contain seven or even more nuclei in each. Soon afterwards, however, they disappear and only single yolk cells are met with. All this time the yolk-cells have been penetrating deeper and deeper into the yolk and thus become distributed throughout the egg.

The morphological position of the primary and secondary yolk cells is discussed below.

6—General Discussion on Gastrulation, with a New Theory of Multi-phased Gastrulation Among Insects

a. General Discussion—The understanding of the nature of insect gastrulation is bound up firstly with the interpretation of the nature and morphological position of the yolk cells, and secondly with the mode of origin of the mid-gut epithelium. On the answer to these questions will naturally depend what process or processes in insect development are to be regarded as corresponding to gastrulation among other animals. In general, gastrulation is characterized by the following features :—

- (i) It is a process by which the single-layered embryo becomes two-layered, the ectoderm and the endoderm being thus laid down. Soon afterwards, the embryo becomes three-layered owing to the differentiation of the mesoderm from the endoderm. Thus, differentiation of the endoderm precedes that of the mesoderm.
- (ii) Gastrulation may occur by any of the following four processes or their combinations, viz., by emboly or invagination, by epiboly or overgrowth, by delamination, and lastly, by inward migration of cells from one pole of the gastrula. In alecithal eggs gastrulation is generally a simple, clear process, usually brought about by epiboly, and the usual sequence of events is neither much delayed nor much disturbed. On the other hand, in eggs which are rich in yolk, as for example insect eggs, the whole process of gastrulation becomes complicated owing largely to the fact that the presence of yolk tends to retard general cell-activity.

There exists to-day two main views regarding the nature of gastrulation among insects.

1. A true blastula stage occurs in insects and is formed when the cleavage cells reach the egg-periphery. Gastrulation occurs afterwards when the inner layer is differentiated. The yolk cells have no morphological significance so far as the germ layers are concerned. The endoderm generally arises as a bipolar structure—at

the blind ends of the stomodaeal and proctodaeal invaginations. This view was advanced by KOWALEWSKY (1886) and is supported by WHEELER, NUSBAUM, and FULINSKI, STRINDBERG (in a slightly modified form), PHILIPTSCHENKO, and others, and more recently by MANSOUR (1927), and EASTHAM (1927 and 1930).*

2. No true blastula stage occurs among insects. The so-called blastula is in reality a post-gastrula stage. The supporters of this view differ among themselves in certain points and their views can be classed into two groups, viz.,

(a) Older biphased gastrulation theory. This was first clearly put forward by HEYMONS (1895 and 1901). According to him, insect gastrulation occurs in two phases of which the first is represented by the separation of the primary yolk cells (intravitelline separation), and the second by the inward migration of secondary yolk cells from the primary epithelium (circumpolar separation). The differentiation of the inner layer is not regarded as a part of gastrulation. PATTEN was also of this opinion and says (1890, p. 368), "That the median furrow of insects is merely an ontogenic adaptation is sufficiently evident from the fact that it may be present or absent in closely related forms". According to this view, the yolk cells alone represent the endoderm. But since they eventually degenerate, there is, among the Pterygote insects, no true endoderm sharing in the formation of the adult body. The mid-gut epithelium is of ectodermal origin. This view has been supported by LÉCAILLON (1897-8), SCHWARTZE (1899), and the majority of those authors who regard the mid-gut epithelium as ectodermal in origin.

(b) Newer biphased gastrulation theory. This view also maintains insect gastrulation to occur in two phases. The first phase corresponds to the first phase of the older view discussed above. But the second phase is represented here by the differentiation of the inner layer and the inward migration of the yolk cells from the primary epithelium. Thus, there is, among insects, no blastula stage and therefore no blastoderm. The primary epithelium (or Oberflächenepithel of HIRSCHLER, 1924) is equivalent to the ectoderm which is composed of two potential elements, viz., a provisional membrane epithelium which goes to form the transitory embryonic membranes, and a definitive germ band epithelium which forms the germ band proper. The latter is potentially more than ectoderm since from it there differentiates the inner layer (= endo-mesoderm). The yolk cells represent the provisional primary endoderm. The secondary endoderm, which arises from the inner layer at the blind ends of the stomodaeum and the proctodaeum, goes to form the mid-gut epithelium. This theory, first suggested by WILL (1888), has received support from a number of authors including NOACK (1901), DICKEL (1904), SCHWANGART (1904-1906), and HIRSCHLER (1912, 1924). Although agreeing in fundamentals, these authors differ from one another in details. Thus, SCHWANGART maintained that,

* HENSON (1932) has recently attempted to homologize the stomodaeal and proctodaeal invaginations of *Pieris* embryo with the oral and anal remnants of the blastopore of *Peripatus*. The blastopore of *Peripatus* is formed simultaneously with the differentiation of the endo-mesoderm. The stomodaeal and proctodaeal invaginations of *Pieris*, on the other hand, appear long after the differentiation of the endo-mesoderm (inner layer). HENSON's view, is, therefore, unacceptable.

in the Lepidoptera studied by him, the yolk cells take some share in the formation of the mid-gut epithelium. This conclusion, however, is not accepted by HIRSCHLER who worked on the same group of insects. Again, NOACK, DICKEL, and SCHWANGART believed that in the different groups of insects studied by them, viz., the Diptera, the Hymenoptera, and the Lepidoptera respectively, a shallow invagination occurred during the first gastrulation phase. This was denied by HIRSCHLER, according to whom a gastral groove does not exist in the first gastrulation phase. This latter author has discussed (1924) the newer biphased gastrulation theory in an excellent and convincing manner. He has shown that the primary yolk cells of insects are to be regarded as equivalent to the Annelidan macromeres. The most convincing evidence for this view we get from the embryology of the Strepsiptera (HOFFMANN, 1914; NOSKIEWICZ and POLUSZYNSKI, 1928), as discussed below. Further, in insects, all the three germ layers, viz., ecto-, meso-, and endoderm share in the formation of the completed body, although the share of the endoderm is considerably reduced and modified. This change of reduction and modification has been brought about by the large amount of yolk present in insect eggs. HIRSCHLER (1912) has shown that such modifications are not restricted to insect eggs alone, but also occur in other Arthropods and in various other animal groups, viz., fishes (Teleostians and Selachians), Cyclostomes, Tunicates, and Cephalopods. Phylogenetically, biphased gastrulation is a secondary feature brought about by the large amount of yolk. The process of intravitelline separation of yolk cells, on the other hand, is primary. Its absence is secondary, so that the non-existence of the first gastrulation phase in a few insects should be regarded as a secondary modification.

b. A New Theory of Multi-phased Gastrulation Among Insects—The theory to be discussed is essentially a development of the newer biphased gastrulation theory and has been necessitated by certain facts recently discovered by myself and other authors. It also attempts, in part, to combine the already described older and newer biphased gastrulation theories. From both of these, however, it differs in the fact that insect gastrulation is no longer to be regarded as a process occurring in only two main periods of activity but in several. This, it need hardly be pointed out, has been brought about by the large amount of yolk present in insect eggs. The whole process of gastrulation has been extremely elongated in time and the time relations of the various phases of the process are profoundly modified. The main theme of this theory will be developed below with reference to what occurs in *Locusta migratoria* and then compared with other insects. Gastrulation in *Locusta migratoria* occurs, according to this view, in the following stages :—

First Phase—Cleavage. Intravitelline separation. (This phase of gastrulation occurs by modified epiboly and results in the differentiation of the primary endoderm represented partly by the primary yolk cells.)

Second Phase—First ventral groove (corresponding to part of gastral groove). Yolk cell membrane (corresponding to part of evanescent primary endoderm). Multi-layered condition of the germ band is an indication of activity during this phase.

Third Phase—Second ventral groove (corresponding to part of gastral groove). Formation of inner layer (endo-mesoderm).

Fourth Phase—Secondary yolk cells (corresponding to part of secondary endoderm ; transient).

Thus, in *Locusta migratoria* there are four main periods of activity into which the whole process of gastrulation is divided. These periods are fairly sharply demarcated from one another, although they sometimes overlap, as for example in the formation of the secondary yolk cells which is spread over a long period. Nevertheless, one can roughly speak of gastrulation as occurring in four phases. The first, third and, to some extent, the fourth phase occur in all insects, but the second phase has, so far, been recorded in a few insects only. However, the phenomena occurring during this last phase have an important bearing on gastrulation. Evidence has recently accumulated which shows that these processes are by no means confined to *Locusta migratoria*. Thus, the presence of a yolk-cell membrane in *Carausius* (*Dixippus*) *morosus* (HAMMERSCHMIDT, 1910 ; LEUZINGER, 1925 ; LEUZINGER and WIESMANN, 1925), and of a first ventral groove in *Pieris rapae* (EASTHAM, 1927) and in *Calandra granaria* (INKMANN, 1933, "Ventralrinne") lends support to the importance of this phase of gastrulation. The formation of a groove soon after the differentiation of the inner layer of *Gryllotalpa* (KOROTNEFF, 1885) indicates the presence of a phase of gastrulation activity which falls between the third and fourth phases of *Locusta migratoria*. It is thus clear that insect gastrulation occurs in three or more main phases, their actual number varying in different insects. The term "multi-phased gastrulation" is, therefore, proposed to cover all these phases. Since it is very likely that more exact work on the early stages of insect embryology will bring to light structures such as supernumerary ventral grooves, etc., which may represent some phase of gastrulation, I have refrained from giving definite names to the various phases of gastrulation as observed in *Locusta migratoria*. The terms tentatively adopted depend on their time relations in this insect. Nevertheless, it has been considered necessary to make certain changes in the nomenclature of some structures formed during the early embryonic period of insects. I propose the adoption of the following terms (some of which are already in use) :—

Primary Epithelium (corresponding to the so-called blastoderm)—Its German equivalent "Oberflächenepithel" was first suggested by HIRSCHLER (1924). Some authors still regard a true blastula to occur among insects and the primary epithelium as the blastoderm. The term "primary epithelium" is non-committal, and hence to be preferred.

Primary Yolk Cells—These are the cells that are left behind in the yolk as a result of intravitelline separation. They are a part of the primary endoderm and are to be compared to the Annelidan macromeres.

First Ventral Groove—Is part of the so-called gastral groove which is formed afterwards. Has been recorded in *Locusta migratoria*, *Pieris rapae*, and *Calandra granaria*. In *Pieris* it is continuous with the second ventral groove but in the other two insects it is not.

Second Ventral Groove—This is equivalent to the gastral groove of most authors and the mesodermal groove of LÉCAILLON (1898). Present in many insects but absent in others. Is part of the gastral groove.

Third Ventral Groove—This has been recorded by KOROTNEFF (1885) in *Gryllotalpa*, and is formed after the differentiation of the inner layer. Hence it is not equivalent to the first and second ventral grooves but comes after them, although in *Gryllotalpa* both the first and second ventral grooves are absent.

Inner Layer—This is the layer which is differentiated from the primary epithelium on the inside, by any of the various methods of gastrulation. It is equivalent to the "unteres Blatt", mesoderm, endo-mesoderm, hypoblast, etc., of other authors and is, potentially, an endo-mesoderm. Since some authors still regard it as pure mesoderm, the non-committal term "inner layer" is adopted here.

Secondary Yolk Cells—These are cells given off into the yolk after the first gastrulation phase, i.e., after the formation of the primary yolk cells. They are part of the secondary endoderm.

The development of the mid-gut epithelium of *Locusta migratoria* has been studied by me and the full account will appear in the second paper of this series. It is shown to be ectodermal in origin. I have also discussed there the nature of the insect endoderm. Since a consideration of this question is of importance in dealing with gastrulation, I shall describe very briefly the conclusions I have arrived at. These are: (1) that the insect endoderm consists of primary and secondary portions (the primary and secondary endoderm of the German authors); (2) that the definitive insect mid-gut epithelium, no matter in what manner arising, is, in the majority of insects, a secondary phenomenon. It may arise from pure ectoderm, from the inner layer (secondary endoderm) or from the ectoderm plus secondary yolk cells (= secondary endoderm). In the Strepsiptera alone (HOFFMANN, 1914; NOSKIEWICZ and POLUSZYNSKI, 1928) do the *primary* yolk cells form a transient mid-gut ("primary mid-gut" of HOFFMANN).

7—The Formation of the Embryonic Membranes

The formation of the embryonic membranes in *Locusta migratoria* begins almost simultaneously with the differentiation of the inner layer. It is brought about by the inward folding of the lateral borders of the germ band ventrally. At about the 42 hour stage there appears the cephalic fold of the embryonic membranes (figs. 32 and 34, Plate 34, and fig. 42, Plate 35). Afterwards, at about the 46 hour stage, a similar fold appears at the caudal end of the embryo (fig. 36, Plate 2, and fig. 43, Plate 35). Meanwhile, the region of the embryo lying between the cephalic and the caudal folds also begins to grow round and forms the lateral folds (fig. 35, Plate 34). The cephalic and the caudal folds travel towards each other and eventually fuse with the lateral folds which also close ventrally. At about the 50 hour stage, the formation of the embryonic membranes is complete. In the early stages of membrane formation, the inner membrane or amnion cannot be

clearly distinguished from the embryonic region (fig. 35, Plate 34). Later on, however, when the folds have fused together on the ventral side of the embryo, this distinction is possible. In this way, two embryonic membranes are formed, viz., the amnion and the serosa. The amnion is differentiated from the embryonic region and consists of elongated cells with elongate nuclei. The serosa is a continuation of the extra-embryonic portion of the primary epithelium and is composed of typical pavement cells which are polygonal in shape. Their nuclei, like those of the amnion, are disk-shaped, being flattened at right angles to the egg-periphery and present a lenticular appearance when viewed from the edge. They measure about 24–28 μ in diameter, and are generally irregularly distributed. At the posterior pole of the egg, however, (and later on at the anterior pole also), the serosal nuclei are closely crowded together to form a circular area of about 380 μ in diameter (fig. 37, Plate 34). In this area the nuclei decrease in size from the periphery towards the centre. The peripheral nuclei measure about 16 μ in diameter and the central ones about 10 μ or less. The two membranes are at first closely applied to each other (fig. 34, Plate 34). Soon, however, they separate at the sides and a thin layer of yolk comes to lie between them (fig. 36, Plate 34, and fig. 38, Plate 35). But in the centre they still remain closely applied to each other and no yolk is seen between them in this region.

With regard to the position of the embryo after membrane formation, the majority of insects fall into one of the two groups, viz., the superficial germ-band type and the submerged type. In the former, the amnion and the serosa are closely applied to each other so that there is no yolk in between them. In the latter, the two membranes are separated by a layer of yolk—the germ band is thus, so to speak, submerged into the yolk. The Acrididae occupy an intermediate position between these two types. In *Locusta migratoria* the amnion and the serosa are separated at the sides by a fairly thick layer of yolk which may even contain yolk cells. But in the middle, they are either closely applied to each other or are separated only by a very thin layer of fluid in which there are no yolk particles. GRABER (1888, *a, b*) has described the same condition in another Acridid *Stenobothrus*. This intermediate condition has not been described in other insects.

8—Changes in the External Form of the Embryo and the Primary Segmentation of the Inner Layer

GRABER (1888, *b*, 1890) gave a good account of the changes in the external form, etc., of the embryo of *Stenobothrus*. More recently, NELSEN (1931, 1934, *a*) and SLIFER (1932, *a*) have described the external changes of form in the embryo of *Melanoplus differentialis*, but their accounts of the early stages are incomplete.

The germ disk of *Locusta migratoria* starts forming as a thickening of the primary epithelium cells lying at the posterior pole of the egg, slightly on the ventral side (fig. 25, Plate 33, and figs. 39–41, Plate 35). Thus, a round, concave germ disk arises which is composed of large, prismatic cells with small, disk-shaped nuclei

measuring approximately $8\ \mu$ in diameter. The cells of the extra-embryonic primary epithelium are more or less spindle-shaped. Their nuclei measure about $16\ \mu$ in diameter and are thus about twice the size of those of the embryonic cells. The germ disk afterwards begins to elongate at its ventro-anterior end along the ventral side of the egg. In about the 42 hour stage, two distinct regions of the embryo can be distinguished—the broad protocephalon and the narrow, elongated protocorm (fig. 42, Plate 35). At this stage the two regions are nearly equal in length but differ in width. The protocephalon measures about $464\ \mu$ across, and the protocorm only about $250\ \mu$. The latter tapers slightly at its extremity. The total length of the germ band, at this stage, is about $665\ \mu$. (In exceptional cases, the protocorm may arise not from the edge of the germ disk as usual, but from its middle). A notch in the mid-frontal region of the embryo is seen at this stage, and is due to the sides of the embryo in this region developing faster than the middle. It, however, soon disappears.

The protocorm rapidly elongates and, in about the 46 hour stage is already about twice as long as the protocephalon (fig. 43, Plate 35). External body segmentation of the embryo has not yet begun, but the middle region of the protocorm shows, on either side, a slight swelling. These swellings, however, are of no segmental significance. The inner layer, at this stage, extends all along the germ band, except the extreme frontal region of the protocephalon.

In about the 50 hour stage (fig. 44, Plate 35) the inner layer begins to show signs of segmentation especially in the thoracic region. At the anterior end it is swollen and has a notch in front. The rudiment of the stomodaeum is also seen. The head lobes are greatly developed and are bent ventrally and medianally at the edges.

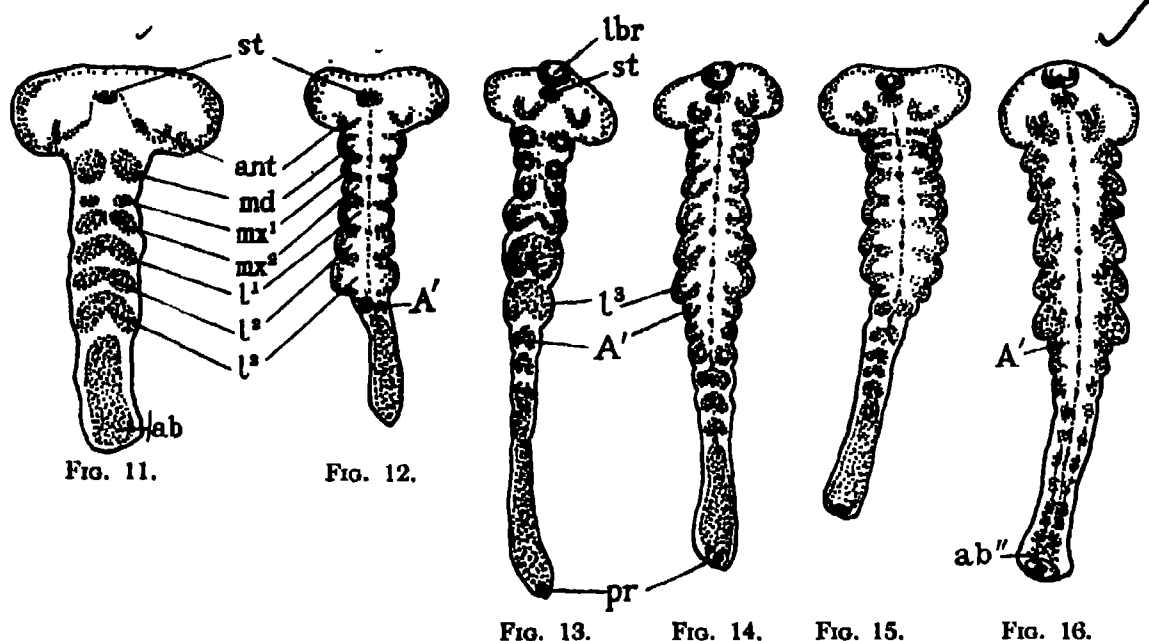
In a slightly older stage, the germ band is seen to have divided into four primary segments—a protocephalic and three protocormic elements. The inner layer has also undergone a segmentation into four parts which roughly correspond to the external primary segmentation. The antennary rudiments have made their appearance. No other appendages are seen as yet.

In a later stage, viz., the 52 hour stage (fig. 11), the rudiments of the three jaw and the three thoracic appendages are seen to have been differentiated in the inner layer and the latter is divided into two lateral halves in each of these segments. Externally, however, these segments are not yet established. The primary external segmentation is less distinct than before. Thus, the definitive segmentation of the inner layer precedes that of the ectoderm.

In the 53 hour stage (fig. 12), the definitive external segmentation of the body becomes evident. The abdomen is still unsegmented, although the rudiments of the first abdominal appendages are seen as a pair of thickenings of the inner layer. By about the 75 hour stage, the whole of the abdomen becomes externally segmented into eleven segments and thus the definitive body segmentation is established.

It should be pointed out that in the 59 hour stage (fig. 13) the embryo passes through an extremely long and thin stage, when it measures about $2.5\ \text{mm}$ in length and about $0.23\ \text{mm}$ across the metathorax. Subsequently, it undergoes an actual

shortening in length, and becomes stouter as already seen in the 60 hour stage (fig. 14), when it measures about 2.3 mm in length and 0.35 mm across the metathorax. After this, the absolute length of the embryo increases but the length in proportion to its width decreases, so that the embryo looks short and stumpy. After blastokinesis the ratio of length to width again increases. Shortly before the beginning of blastokinesis the eye-pigment first makes its appearance as a faint orange-coloured area at the posterior border of the large rudiments of the compound



FIGS. 11-16—Early germ bands. A', first abdominal appendage; ab., abdomen; ab'', eleventh abdominal segment; ant., antenna; l¹-l³, first to third thoracic legs; lbr., labrum; md., mandible; mx¹, first maxilla; mx², labium; pr., proctodaeum; st., stomodaeum.

FIG. 11—Germ band 52 hours old. The rudiments of the jaw appendages and the thoracic legs are seen. × 45.

FIG. 12—Germ band 53 hours old. Definitive external segmentation of the body has begun. The rudiments of the first abdominal appendages are seen. × 30.

FIG. 13—Germ band 59 hours old. It is very long and thin. × 30.

FIG. 14—Germ band 60 hours old. It is becoming shorter and stouter. × 30.

FIG. 15—Germ band 65½ hours old. × 30.

FIG. 16—Germ band 70 hours old. The eleven abdominal segments are clearly seen. × 30.

eyes. As pointed out by SLIFER (1932, a), after blastokinesis "the state of development of the hind femora is perhaps the best criterion to be used in estimating the age of such embryos".

It will be seen from the above account that the definitive body segmentation of the locust embryo is preceded by a transient primary segmentation into four "macro-meres" or "macro-somites", exactly like what obtains in *Donacia* (HIRSCHLER,

1909). This primary segmentation is shared by the inner layer also. It was first observed in insect embryos by AYERS (1884) in *Oecanthus*. Afterwards, GRABER (1888, b), NUSBAUM (1889), KULAGIN (1898), HIRSCHLER (1909), SEIDEL (1924) and GRANDORI (1932) have reported it in several other insects belonging to different orders. In the Acridid *Stenobothrus*, GRABER (1888, b) was able to observe primary segmentation in the inner layer only, and not in the external body form. NELSEN (1931, 1934, a) and SLIFER (1932, a) make no mention of the primary segmentation in *Melanoplus differentialis*.

It is with great pleasure that I take this opportunity of expressing my sincere thanks to Professor J. STANLEY GARDINER, F.R.S., for accommodating me in the Zoological Laboratory, Cambridge. To Dr. A. D. IMMS, F.R.S., I owe a deep debt of gratitude for his kindly guidance and friendly counsel throughout the progress of this work. Part of this work was done in the Kaiser Wilhelm-Institut für Biologie, Berlin-Dahlem, during the summer of 1934. To Professor RICHARD GOLDSCHMIDT I am grateful for giving me a table in the Institute. I am indebted to the Alexander von Humboldt-Stiftung, Berlin, for a grant to enable me to continue this work in Berlin. To Fräulein Dr. ELISABETH HÖNER and to Dr. H. W. LISSMANN I wish to express my thanks for their help in the preparation of some of the illustrations. Finally, I would like to thank Professor L. E. S. EASTHAM for suggesting some improvements in the text.

IV—APPENDIX.

While this work was in the press there has appeared an important paper by the late A. J. THOMAS (1936) on the embryonic development of *Carausius morosus* (Phasmidae). The following remarks are made on this paper, so far as it touches the present work.

Formation of the Inner Layer.—The inner layer of *Carausius* arises, according to THOMAS, by the method of invagination, i.e., by proliferation from the roof of a mid-ventral groove, called by him the "gastral furrow". It is interesting to note that previous workers on the same insect (HAMMERSCHMIDT, 1910; STRINDBERG, 1914; and LEUZINGER, 1925) describe the inner layer as arising by immigration, without the formation of a mid-ventral groove.

A point of extreme interest is the discovery by THOMAS in *Carausius* of an "anterior ventral groove" which precedes, in time, the "gastral furrow". The latter is formed after, and as a continuation of, the former. The "anterior ventral groove" of *Carausius* recalls a similar structure described by EASTHAM (1927) in *Pieris*, with this difference, that whereas in *Pieris* the cells budded off from the roof of this groove degenerate, in *Carausius* they form the yolk-cell membrane (*vide* below). Needless to say, the two grooves in *Carausius* correspond to the "first" and "second" ventral

grooves of *Locusta* and furnish an additional support to the idea of multi-phased gastrulation among insects. It forcefully confirms my previous conclusions that more exact work on the early stages of insect embryology will bring to light the existence of structures such as supernumerary ventral grooves, etc., which may represent some phase of gastrulation.

Yolk-Cell Membrane.—THOMAS confirms the presence of the yolk-cell membrane in *Carausius morosus* described by the previous writers cited above. He also agrees with them in regarding it as the primary endoderm which "might represent the vestiges of an ancient mid-gut epithelium which was primitively formed from the yolk cells". But his statement (p. 502) that this membrane was regarded by HAMMERSCHMIDT (1910) and LEUZINGER (1925) to give rise to the lining of the mid-gut, is inaccurate. LEUZINGER and WIESMANN (1925) clearly state (p. 102) that it only serves as a sliding path ("Gleitbahn") for the compensatory or replacement cells ("Ersatzzellen"—secondary endoderm) which ultimately form the mid-gut epithelium.

The most important part of THOMAS's finding in regard to the yolk cell membrane is the determination of its precise mode of origin, which was not so clear to the older authors. It arises as a proliferation of cells from the middle of the germ band, and is preceded, in point of time, by the first ("anterior") ventral groove. Thus, it is clearly demonstrated in *Carausius* that the first ventral groove is a kind of gastrulation groove whose existence is related to the formation of the primary endoderm (yolk-cell membrane). Both these structures are formed in *Locusta* where, however, they make their appearance simultaneously. The second ventral groove of *Carausius* is related to the formation of the inner layer. The example of *Carausius* thus conforms to the classical mode of animal gastrulation in which at first the ectoderm and endoderm are differentiated and later the mesoderm, the only difference being in the formation of two gastral grooves, instead of one, in *Carausius*.

The recent paper of DRUMMOND (1936) on *Ephestia Kühniella* may also be mentioned here. In this apparently inexact and superficial work, she claims that the inner layer arises from a middle plate which is later overgrown by the lateral plates; no mid-ventral groove is formed. This is in contradiction to the findings of SEHL (1931) on the same insect. He shows (p. 582) the formation of a clear gastral groove ("Primitivrinne") from the roof of which the inner layer arises. It is strange that DRUMMOND makes no reference to the important work of SEHL.

Finally, I should take this opportunity of mentioning the occurrence of certain supernumerary ventral grooves which have come to my knowledge since the paper was communicated to the Society. GRABER (1889) has shown that in *Calliphora* three parallel gastral grooves occur instead of a single one. He regards this as an instance of "lateral" gastrulation. HEYMONS (1895) has shown a similar condition to obtain in *Periplaneta*. The theory of multi-phased gastrulation propounded in this paper had mainly contemplated the elongation of gastrulation in time and its

subsequent splitting up into several phases. The two examples cited above would seem to show that the elongation of the gastrulation phase can occur not only in time but also in space. The term "multi-phased gastrulation" should, therefore, be extended to include both of these cases.

V—SUMMARY

For the study of embryonic development, eggs were incubated at a constant temperature of 33° C, and in moist sand.

The earliest cleavage stage described is the 4-cell stage. The cleavage cells lie near the posterior pole of the egg where, presumably, fertilization takes place.

The cleavage cells divide most rapidly during the 7 to 10 hour period, after which there is a progressive slowing down. The rate of migration of cleavage cells towards the anterior pole of the egg rapidly rises after the 10 hour stage. Migration takes place along the egg-periphery.

The formation of the primary epithelium (= "blastoderm") first takes place at the posterior end of the egg and thence travels anteriorly. Intravitelline separation occurs, thus giving rise to primary yolk cells.

At the 30 hour stage, a shallow groove termed the "first ventral groove" is formed in the mid-ventral line of the anterior portion of the germ disk. It lasts for about 4 hours or less and is not connected with the differentiation of the inner layer. At the same stage, a yolk-cell membrane is formed on the ventral side of the embryo. It disappears within four hours.

The "second ventral (gastral) groove" appears at the 42 hour stage and lasts for 3-4 hours. The inner layer is proliferated from its roof.

The structure of the yolk, secondary yolk cleavage, etc., are described. Secondary yolk cells may arise either by multipolar migration inwards or by division of primary epithelium cells, the latter being a new mode not so far recorded among insects.

A new theory of multi-phased gastrulation among insects is proposed and discussed. It regards insect gastrulation as occurring in several phases.

The homologies of the primary epithelium, primary and secondary yolk cells, ventral grooves and the inner layer are discussed.

Both the amnion and the serosa are present and arise in the usual way. Their relation to the germ band and to each other is such that there obtains a condition which is intermediate between the superficial and the submerged types of germ bands.

Changes in the external form of the embryo are described. A primary segmentation of the body (involving both the ectoderm and the inner layer) into four elements precedes the definitive one.

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VII—EXPLANATION OF PLATES

All figures, except where otherwise stated, are from camera-lucida drawings. The age of the stages referred to signify the period after egg-laying when kept at a constant temperature of 33° C and on moist soil (humidity near saturation).

Lettering

a., extent of distribution of cleavage cells at the 8 hour stage ; *A'.-A².*, first and second abdominal appendages ; *ab.*, abdomen ; *ab''.*, eleventh abdominal segment ; *am.*, amnion ; *am.v.*, amniotic cavity ; *ant.*, antenna ; *c.*, cells proliferating from the roof of the first ventral groove ; *c.d.*, cell clusters ; *cd.*, caudal end ; *c.c.*, cleavage cells ; *ceph.*, cephalic end ; *deg.y.c.*, degenerating yolk cells ; *ect.*, ectoderm ; *em.mem.*, embryonic membranes ; *em.obr.*, embryonic portion of the primary epithelium ; *e.w.*, egg-wall ; *ex.obr.*, extra-embryonic portion of the primary epithelium ; *f.*, negative images of fat globules ; *f.v.g.*, first ventral groove ; *g.b.*, germ band ; *g.d.*, germ disk ; *h.l.*, head lobes ; *in.l.*, inner layer ; *I'.-I³.*, first to third thoracic legs ; *lbr.*, labrum ; *l.c.*, large cell ; *m.c.*, micropylar canal ; *md.*, mandible ; *micr.*, probable micro-organisms ; *mx¹.*, first maxilla ; *mx².*, second maxilla or labium ; *nu.*, nucleus ; *obr.*, primary epithelium ; *pcl.*, protocephalon ; *per.*, protocorm ; *per.*, periplasm ; *pr.*, protoplasm ; *p.y.c.*, primary yolk cells ; *s.*, swellings on either side of the second ventral groove ; *ser.*, serosa ; *st.*, stomodaeum ; *s.v.g.*, second ventral groove ; *s.y.c.*, secondary yolk cells ; *vac.*, vacuole ; *y.*, yolk ; *y.c.m.*, yolk-cell membrane ; *y.c.s.*, yolk-cell syncytia ; *z.*, yolk particles probably in a phase of digestion ; I, II, III, IV, primary segments of germ band.

PLATE 33.

FIG. 17—Portion of a longitudinal section near the posterior pole of a 13 hours-old egg. The cleavage cells are near the egg-periphery. × 370.

FIG. 18—The same of an 18 hours-old egg. The cleavage cells have reached the egg-periphery where they flatten out temporarily. Some yolk particles, probably in a phase of digestion, can be seen at *z.* × 370.

FIG. 19—Transverse section near the posterior pole of an 18 hours-old egg. × about 37.

FIG. 20—Portion of a longitudinal section near the posterior pole of a 21 hours-old egg, showing the tendency of the cleavage cells to form groups. The cells have lost their flattened form (*cf.* fig. 18) and are again rounded or oval. × 370.

FIG. 21—The same from a 23 hours-old egg. The cells have formed a continuous layer on the egg-periphery at the posterior end of the egg. A secondary yolk cell is seen migrating inwards from the periphery (*cf.* fig. 24). × 370.

FIG. 22—Transverse section near the posterior pole of an egg about 23 hours old, showing the primary epithelium on the ventral side. × about 37.

FIG. 23—Portion of a longitudinal section of an egg of the same stage as in fig. 20, showing a cell near the anterior end of the egg. Compare this cell with those in fig. 20. × 370.

FIG. 24—Portion of a longitudinal section near the posterior pole of an egg about 23 hours old, showing two stages in the formation of secondary yolk cells by migration (*cf.* fig. 21). × 370.

FIG. 25—Transverse section near the posterior end of an egg 28 hours old. × about 100.

FIG. 26, *a, b*—(*a*). Part of the ventral (embryonic) region in fig. 25 more magnified. Note the flattening of yolk cells close to the germ disk. They are on their way to form the yolk-cell membrane. × 370. (*b*). The same, but showing the region where the embryonic and extra-embryonic regions of the primary epithelium meet. × 370.

FIG. 27—Portion of a transverse section near the posterior end of an egg 30 hours old, showing the first ventral groove, the yolk-cell membrane and the multi-layered condition of the germ band. × about 167.

FIG. 28—Portion of the same more magnified, showing the first ventral groove and the rounded cells proliferating from its roof. × 370.

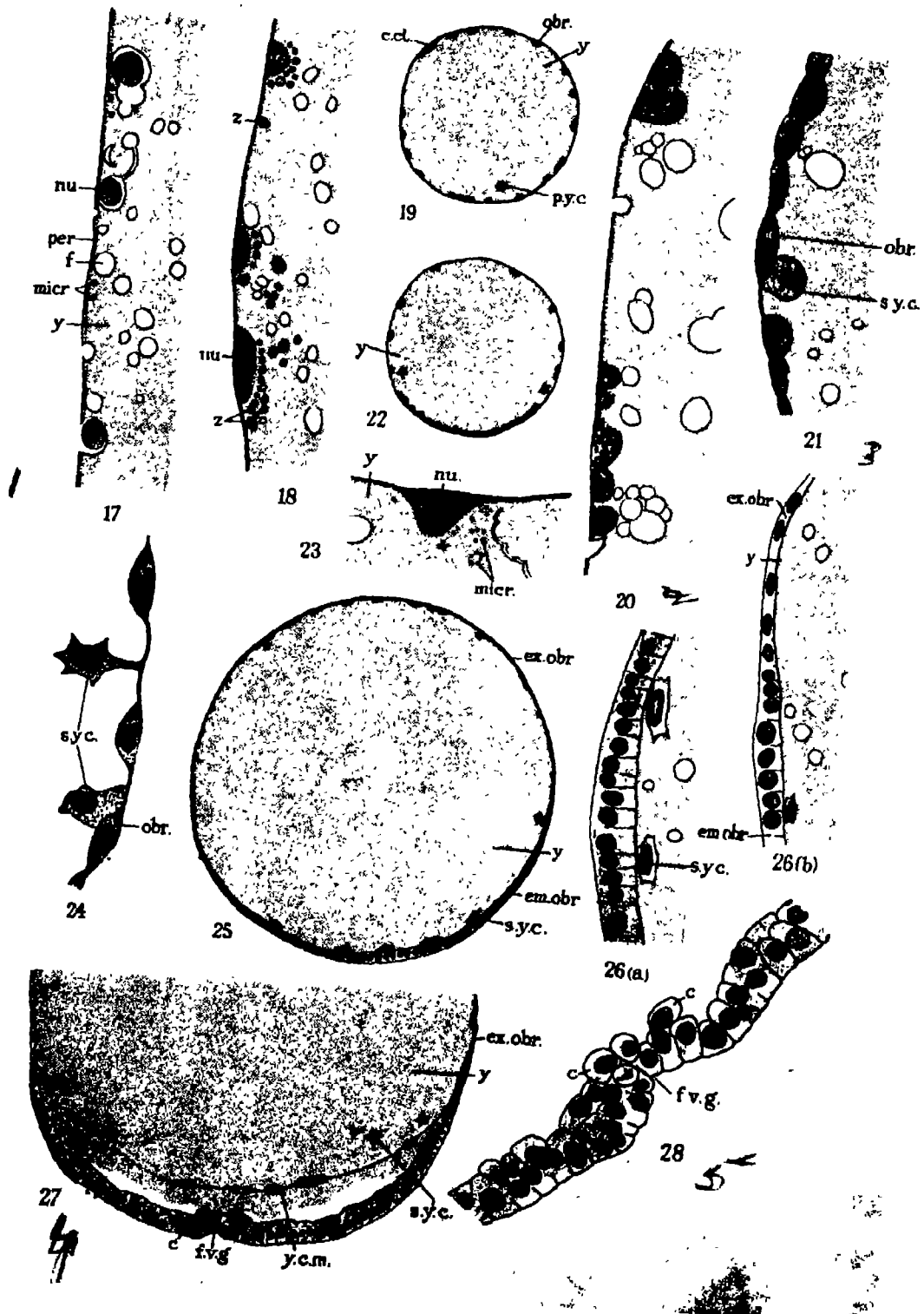


PLATE 34.

- FIG. 29—Portion of a longitudinal section of the posterior end of an egg 30 hours old, showing the first ventral groove and the yolk cell membrane. \times about 92.
- FIG. 30—Portion of a longitudinal section of an egg 40 hours old, showing the extra-embryonic region of the primary epithelium and the yolk-cell syncytia. \times 370.
- FIG. 31—Portion of a transverse section of the posterior end of an egg about 34 hours old, showing the germ band region. Note the absence of a single-layered condition in the germ band. A very large cell is seen on the left; it is not a genital cell. Some of the degenerating yolk cells are seen. \times 210.
- FIG. 32—Transverse section of the posterior end of an egg about 42 hours old, passing across the hind region of the protocephalon, and showing the embryonic membranes and the inner layer. \times about 92.
- FIG. 33—Portion of a transverse section across the middle region of a 45 hours-old germ band, showing the second ventral groove and the differentiation of the inner layer. \times 520.
- FIG. 34—Portion of the same as in fig. 32 more magnified. \times 230.
- FIG. 35—Portion of a transverse section of the posterior end of an egg about 45½ hours old, passing through the middle of the germ band. Membrane formation is not yet complete in this region. \times 155.
- FIG. 36—Transverse section across the posterior region of the protocorm of a germ band about 46 hours old, showing the thick inner layer. \times 170.
- FIG. 37—Scrota at the posterior pole of an egg about 40 hours old. The cells at the pole form a circular area of comparatively small and closely packed nuclei. \times 95.

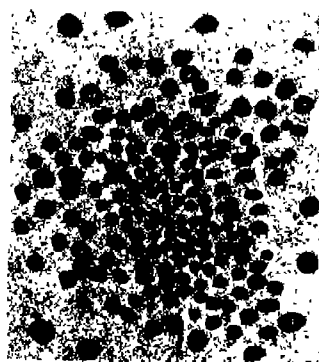
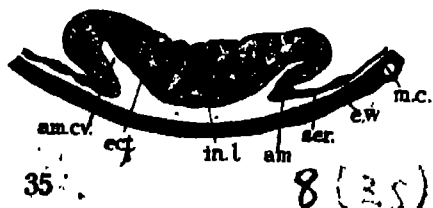
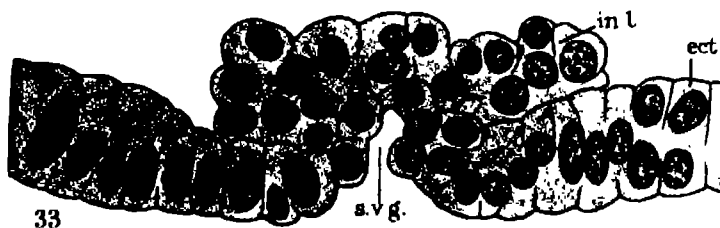
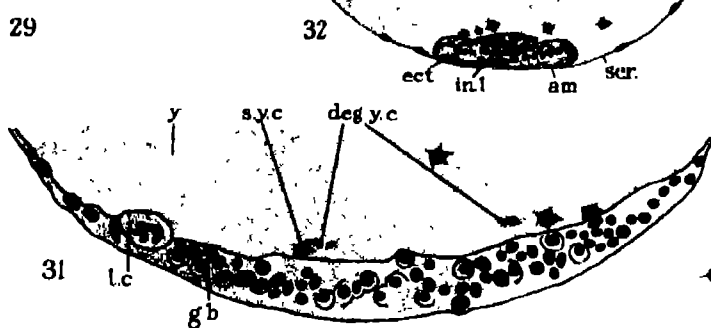
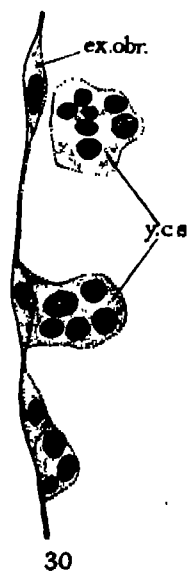
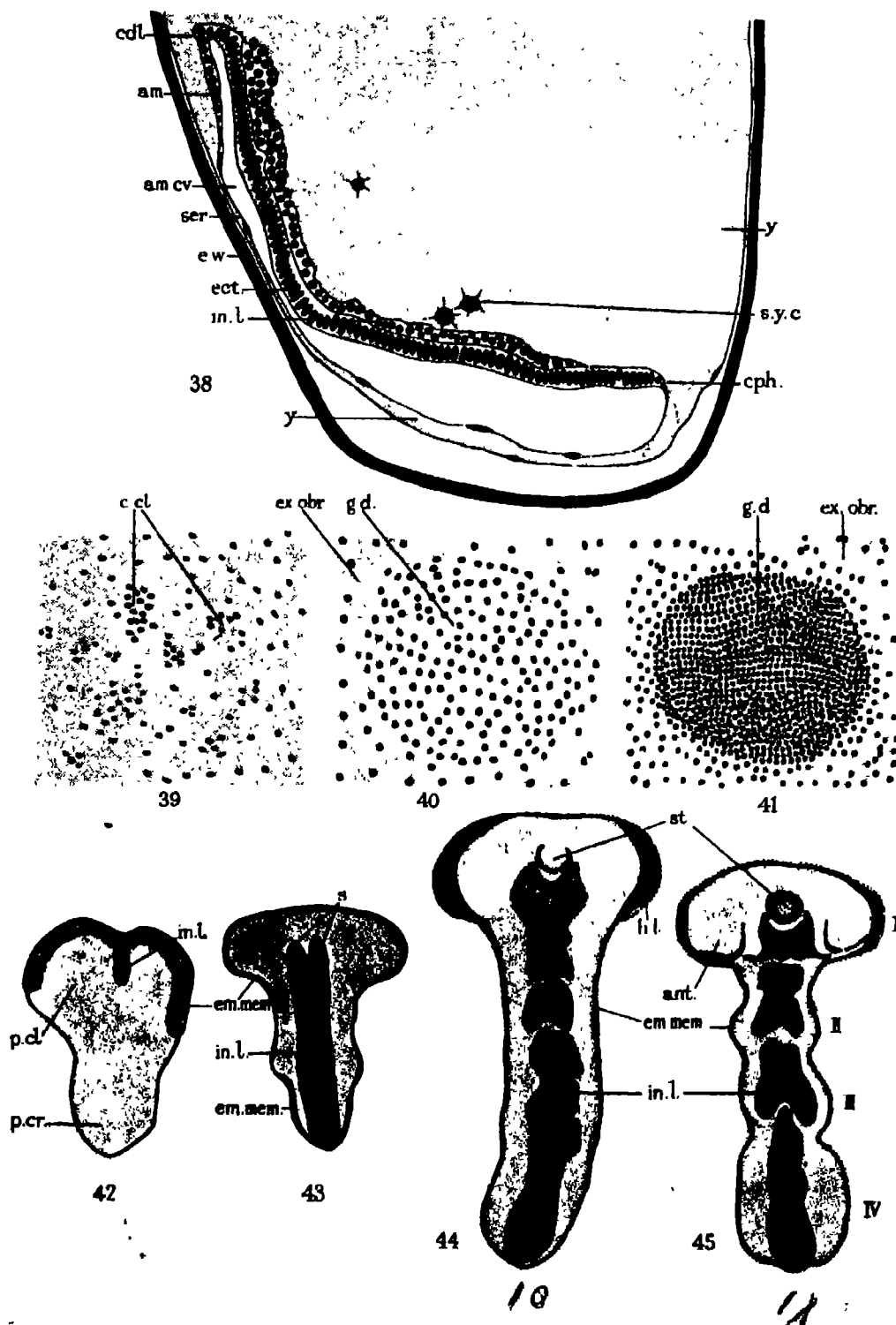


PLATE 35.

- FIG. 38—Longitudinal section of the posterior end of an egg 46 hours old, passing medianally across the germ band. $\times 170$.
- FIG. 39—Surface view of the posterior end of an egg 21 hours old, after removal of the egg-wall, showing groupings of cleavage cells prior to the formation of the germ disk. $\times 56$.
- FIG. 40—The same from a 22 hours-old egg, showing the germ band distinctly marked out and its component cells arranged more or less regularly. $\times 56$.
- FIG. 41—The same from an egg 28 hours old. $\times 56$.
- FIG. 42—Germ band 42 hours old. The embryonic membranes have been formed at the cephalic end, but the caudal end is still free from them. The second ventral groove is beginning to form at the cephalic end. $\times 56$.
- FIG. 43—Germ band about 46 hours old. The embryonic membranes are seen at both the cephalic and the caudal ends, but the middle region of the germ band is still free from them. The area of inner layer differentiation has travelled backwards and reached the caudal extremity. The apparent bifurcation of the inner layer in the cephalic region represents the swellings of the germ band on either side of the second ventral groove. Note also the two lateral bulges in the middle of the protocorm. $\times 56$.
- FIG. 44—Germ band about 50 hours old. The formation of the embryonic membranes is complete. The inner layer is beginning to segment. Note the stomodaeal rudiments and the specially well-developed head lobes. $\times 56$.
- FIG. 45—The same but a slightly older stage. The germ band has divided into four primary segments (I-IV) and the segmentation of the inner layer roughly corresponds to this. The antennary rudiments are seen. $\times 56$.



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XI—THE CROONIAN LECTURE

Sexual Periodicity and the Causes Which Determine It

By F. H. A. MARSHALL, *F.R.S., Cambridge*

(Lecture delivered 18 June—Received 22 July, 1936)

It is a matter of common knowledge that the great majority of animals, both vertebrate and invertebrate, not to mention plants, have a more or less definite season or seasons of the year at which they breed. This time for breeding is generally, though by no means invariably, in the spring and summer, and it is well known that whereas a favourable season as regards warmth and general conditions tends to accelerate breeding an unfavourable one may retard it. So much is known to be generally true, yet the precise factors which determine the sexual season vary in passing from group to group and from species to species or even from breed to breed. WESTERMARCK (1921), confining himself to mammals alone, has pointed out that there is no month of the year at which some species does not have its breeding season, and yet that for the particular species in question the season is most regular. Speaking teleologically, the breeding season is regulated by the times most suitable for the young to be produced and reared. Without disparaging the use of teleological categories which justify themselves as means of generalization and prediction and are very generally used by the naturalist to the great advantage of his work, it is obvious that such a view is no explanation of the physiological causes of sexual periodicity in the individuals of which a species is made up. We still know only a little about these causes. But in view of the general correlation between the seasonal and the sexual cycles it must be assumed that these stand in the relation of cause to effect, unless, indeed, we believe in a pre-established harmony. And nowadays it is not fashionable to believe in pre-established harmonies. Moreover, in countries where conditions are more or less uniform throughout the year, as in some parts of the tropics, *e.g.*, in the Philippines as found by SEMPER (1881), animals of all kinds may breed at any time.* This is not saying that there is no internal rhythm occurring independently of the environment.

In the lower forms of life, especially in those in which there is no nervous system, apart from the alternation of activity and rest associated with the release of the ova and sperms, especially when these are discharged in bulk, the rhythm of reproduction must be controlled metabolically by the direct action of the environment—food, temperature, light, the humidity of the atmosphere, and in water-living animals the chemical composition and hydrogen-ion concentration of the medium. In the higher

* BAKER (1929) states that in the New Hebrides, where the climate and general conditions are fairly constant and the length of the day only varies by about two hours, there is a breeding season in the giant bat (*Pteropus*) and in the bird *Zosterops*. The lizard (*Lygosoma*) breeds all the year round but is most fertile in November and December. The insectivorous bat, *Miniopterus*, also has a season.

forms, certain of the external factors act through the intermediation of the nervous system, and the evidence bearing on this subject I propose to discuss presently. Apart, however, from these factors and from the exteroceptive factors which are dependent on the animals themselves—the stimuli of sight, hearing, smell, and sexual contact, as well as lactation and (in birds) incubation—it is evident that sexual periodicity is conditioned by the general environment, just as all vital processes are so conditioned, and that food supply is of prime importance. This latter fact was recognized by ARISTOTLE who remarks that where the weather is warm and fine and food is abundant sheep may have young twice a year. We see a similar effect, partly, in the increased polyoestrus of so many of the domestic animals. The practice of “flushing” sheep, that is, supplying them with extra food—corn, cake, or turnips, or turning them out on a good new ley, rape or mustard, or merely superior pasture, is well known, not merely to increase the crop of lambs, but to hasten forward the sexual season. Flushing, however, will not bring the ewes “on heat” in the middle of the period of quiescence or anoestrus; it will merely bring them to a higher degree of nutritional activity and so slightly accelerate the time of tupping. The converse effect of a poor nutrition has been described, among others, by PAPANICOLAOU and STOCKARD (1920), who observed the results of underfeeding in producing a disturbance in the oestrous cycle and the rhythm of ovulation in the guinea-pig. Moreover, it is well known that poverty of nutrition during the Great War was often responsible for transient amenorrhoea and irregularity in the menstrual cycle in women. On the other hand, disturbances in the cycle and even complete sterility may be associated with adiposity, as shown both in women and in the domestic animals. Furthermore, the possibility must not be lost sight of that sexual periodicity is affected by the absorption of vitamin E which may be present in greater quantity in the food at certain seasons of the year. Vitamin E, which is found in most concentrated form in certain plants, especially in seeds (oats, corn, etc.) and in green leaves, has been shown by EVANS and his collaborators (EVANS and BURR, 1927) to be essential for spermatogenesis in the male mammal and for embryonic development in the uterus of the female, but as to whether its greater abundance at certain seasons is a factor in breeding periodicity is a matter on which at present there is no evidence.

That the generative functions may be inhibited by faulty nutrition or an inappropriate environment is well shown also by the peculiar case of the marmosets at the Lister Institute. These animals not only failed to breed but suffered from rickets, a disease to which this species in captivity is especially liable. It was found impracticable to supply the necessary vitamin D in cod-liver oil owing to the animals' dislike to it, so ultra-violet rays were used as a substitute, and with complete success, for the marmosets acquired perfect health and bred freely, forming a considerable colony (LUCAS, HUME, and SMITH, 1927). Vitamin D, therefore, or a sufficient supply of ultra-violet rays is a necessary condition for breeding activity, and, as will be shown later, ultra-violet irradiation is a possible factor also in sexual periodicity.

Apart, however, from external factors which condition or regulate breeding, there is undoubtedly in the higher as in the lower animals an inherent reproductive rhythm. Thus, in the dog, at any rate under a state of domestication, the male is capable of breeding at any time of the year, but the female experiences a regular sexual cycle, typically of six months' duration, and which appears to occur independently of any exteroceptive or other external stimuli.* On the other hand, in the nearly related fox, as described by ROWLANDS and PARKES (1936), there is a single annual oestrous cycle, reproduction taking place in the early part of the year.

THE GONADS

Before passing on to the consideration of the stimuli which probably act through the intermediation of the nervous system—and this I propose to do in some detail—it is desirable to state briefly the main known facts concerning the part played by the internal organs in regulating sexual and reproductive rhythm. That the gonads are essential organs in these periodic processes has presumably been recognized ever since castration was first practised. Thus, it is well known that if these organs are removed prior to maturity the sexual cycle never begins, or if this is done after maturity has been reached the cycle stops. Moreover, the gonads in many animals undergo an increase in size at the approach of breeding, and in some, as in most birds and in insectivores amongst mammals, this increase is enormous. In rodents it is often accompanied in the male by the passage of the testes into the scrotum from which they are withdrawn after the sexual season is over. The periodic increase in the size of the accessory sexual organs (prostate, vesiculae seminales, etc.) which, in some mammals such as the insectivores is very great, is also inhibited by castration, and so is the annual growth of certain of the secondary sexual characters such as the antlers in stags. In all the higher animals, to the consideration of which I shall confine myself in this lecture, there is a succession of processes together constituting the breeding phenomena—in birds pairing, nest building, the laying of eggs, incubation, and the feeding and rearing of the young; and in female mammals oestrus, followed by pregnancy and lactation or in some cases, when pregnancy does not supervene, by pseudo-pregnancy. Moreover, in the polyoestrous species of mammals the matter may be complicated by the recurrence of oestrus and the succeeding processes inside the sexual season of the female, so that there is a secondary rhythm within the main sexual cycle.

This brings me to the consideration of the testis and ovary as organs elaborating internal secretions that differ in quantity or in kind in successive phases of the cycle.

The formulation of the idea of internal secretion is usually associated with the name of CLAUDE BERNARD (1859), who applied it to the liver, while at a later date the principle became extended and popularized through the influence of BROWN-SÉQUARD (1889), although his therapeutic observations on which his conclusions

* There is, however, a tendency for the heat periods in the bitch to occur in spring and autumn more than at other seasons.

were based were subsequently discredited. It is interesting to note, therefore, that the fundamental principles on which endocrinology is founded were actually first deduced considerably earlier than the time of BROWN-SÉQUARD, and that this was done as a result of an experimental investigation on the reproductive organs. For it was as far back as 1849 when BERTHOLD published an account of the effects of the removal and transplantation of the testes in fowls. The experiments showed that whereas castration was followed by the loss of masculinity and the cessation of male sexual activity, testicular transplantation in an abnormal position with the ordinary nerve connexions presumably non-existent resulted in the retention of the sexual characters. The conclusion reached, namely, that the gonads elaborate substances, now called hormones, which are responsible for the development of the secondary sexual characters and the initiation and maintenance of the capacity to breed was many years later confirmed and extended as a consequence of experiments conducted on precisely similar lines, both in the male and in the female. Transplantation of the ovaries into abnormal positions in mammals with continuation of the sexual phenomena was effected by KNAUER (1900) and by HALBAN in 1900, and transplantation of the testes with comparable but less conclusive results was done by FOGES in 1903 and by SHATTOCK and SELIGMAN in 1904, and more recently with complete success in various animals by PÉZARD (1911), STEINACH (1920), and many others, sexual potency and the secondary male characters being retained (*cf.* also MONCKTON COPEMAN, 1912). In the meantime, FRAENKEL (1903) showed that the successful implantation of the fertilized ovum depended upon the corpus luteum, and he formulated the theory that the corpus luteum was the essential ovarian organ of internal secretion and was re-formed in different positions in the ovaries with each cycle. In 1905, mainly as a result of an experimental investigation on the dog and the rat, the theory was put forward that there were two ovarian hormones, one being formed by the follicles, or interstitial cells of the ovary (afterwards called oestrin) and the other being produced by the corpus luteum (afterwards called progestin and progesterone). These were believed to be respectively responsible for the two chief stages of the active part of the oestrous cycle, the period of "heat" (prooestrus and oestrus) and the period of pregnancy (MARSHALL and JOLLY, 1905), and this view is now generally accepted as being within limits substantially correct. It has been shown also that the condition of pseudo-pregnancy which occurs under experimental conditions (as after sterile coition) in such animals as the rabbit (this animal normally ovulating only after the orgasm) is also dependent on the corpus luteum (ANCEL and BOUIN, 1910, 1911), and the same is true of the bitch in which pseudo-pregnancy takes place in the non-occurrence of gestation (MARSHALL and HALBAN, 1917). In pseudo-pregnancy the uterus undergoes growth and hyperaemia and glandular development in the same kind of way, but not to the same extent, as during true pregnancy, and there is also mammary growth, followed by the secretion of milk.

Further, at the end of pseudo-pregnancy, animals which experience it may display habits and instincts such as are ordinarily associated with parturition at the end of true pregnancy. Thus the bitch tends to prepare a bed as though for the reception

of young, the rabbit to pluck its breast of fur and to make a nest, and the marsupial cat to clean out its pouch (MARSHALL and HALNAN, 1917, HAMMOND, 1925, HILL and O'DONOGHUE, 1913). These phenomena suggest that the processes occurring at parturition, at least in many species, are functionally correlated with the regression of the corpus luteum which usually occurs at the close both of pregnancy and of pseudo-pregnancy.

In polyoestrous animals (which have a succession of short cycles within the sexual season) the short period of rest which HEAPE (1900) called the dioestrus is now known in most species to be of the nature of an abbreviated pseudo-pregnancy (but not in the rat and mouse), and HAMMOND (1927) and LOEB (1911) have shown in the cow and the guinea-pig respectively that extirpation of the corpus luteum from the ovary reduces this period, a new "heat" or oestrus supervening in about two days after the removal of the gland. Furthermore, SNYDER (1934) and others, by inducing ovulation in the rabbit in the latter part of pregnancy (through injecting urine of pregnancy containing an anterior pituitary principle), have produced a new batch of corpora lutea in the ovaries, and these have been the means of prolonging the pregnancy for an additional ten days. This is further evidence as to the function of the corpus luteum in maintaining pregnancy. It must be mentioned, however, that in some species (such as man and the horse, cat and rat), the corpus luteum can be removed in the later part of pregnancy without terminating it, and there is some evidence that the endocrine function is taken over by the placenta (COURRIER and GROS, 1935, and SELYE, COLLIP and THOMSON, 1935).

It must be emphasized that the change over in the cycle from the oestrous phase to the luteal phase is effected by ovulation, after which the ovarian follicles become converted into the corpora lutea. In such animals as the rabbit and the ferret which do not ovulate spontaneously oestrus may continue for many weeks without any luteal phase. In a somewhat similar way in some animals (*e.g.*, mares and cows) with abnormal cystic follicles which fail to rupture, a nymphomaniac condition of oestrus may go on almost indefinitely. On the other hand, the abnormal persistence of the corpus luteum is associated with failure to experience oestrus.

We must now consider briefly the evidence based upon the extraction and injection of the hormones of the testicle and ovary. The first experiments with definitely potent testicular extracts seem to have been those of PÉZARD (1911), who injected the substances intraperitoneally into capons and obtained growth of the comb. Later, McGEE (1927) and many others confirmed and extended the conclusions, showing that the growth of the accessory male organs, secondary sexual characters, and periodic sexual phenomena could be induced by the injection of testicular substances. Thus, in the male ground squirrel in which the accessory organs atrophy during the non-breeding season, the injection of testicular hormone will restore them to activity (WELLS, 1935). In the female, ALLEN and DOISY (1924) discovered a definite oestrogenic principle in the ovarian follicles, and it was afterwards extracted from the extra-follicular stroma. ALLEN and CORNER (1929) were the first to derive from the corpus luteum a potent extract (progestin) which not only

produced the progestational growth of the uterus in an oöphorectomized female but permitted one which had become pregnant to maintain the foetus in *utero* where it developed until full term.

Thus the main hormones which determine sexual periodicity were obtained in a state of potency, and more recently their chemical composition has been successfully investigated and some of them have been made in the laboratory from other sources than animal tissues.

A whole series of oestrins or oestrus-producing substances are now known to occur (and this has been one of the obstacles encountered in making exact analyses of the chemical factors concerned in the control of the cycle), and several excellent reviews have been written dealing with this subject (DODDS 1934, 1935, A, 1935, B, RUZICKA 1936). Oestrone (ketohydroxyoestrin— $C_{18}H_{22}O_2$) and oestriol (trihydroxyoestrin— $C_{18}H_{24}O_3$) have been isolated from the urine, and also equilenin ($C_{18}H_{22}O_2$) and equiline ($C_{18}H_{24}O_2$). Oestradiol ($C_{18}H_{24}O_2$), the most potent of all the oestrus-producing substances, has been isolated from the ovary and is probably the true ovarian oestrogenic hormone. However, all these substances have been shown to be capable of causing oestrus, but their potency varies considerably. Oestrone is generally taken as the standard, but oestradiol is about five times more potent. The other compounds are less effective. In their composition there is an apparent connexion, as they have the same carbon skeleton as the sterols but differ in the arrangement of the hydroxyl groups round the carbon ring. The active principle of the corpus luteum, named originally progesterin and now known to be a diketone called progesterone ($C_{21}H_{30}O_2$), has also been isolated. Substances having the properties normally ascribed to the testicular hormone have been isolated from male urine; these are androsterone ($C_{19}H_{28}O_2$) and dehydroandrosterone ($C_{19}H_{26}O_2$), while another hormone, with the same physiological properties but a slightly different constitution called testosterone ($C_{19}H_{28}O_2$), has been extracted from the testis itself (DAVID, LAQUEUR *et al.*, 1935). Androsterone is three times as potent as dehydroandrosterone, but testosterone is the most active, being twenty times as effective as dehydroandrosterone.*

* Oestrone was isolated from urine by DOISY (1929), BUTENANDT (1929), and DINGEMANSE and LAQUEUR (1930). Oestriol was also isolated from urine by MARRIAN (1930). SCHWENK and HILDEBRANDT (1933) obtained oestradiol, which is a dihydroderivative of oestrone, by reduction of the ketone group to a secondary alcohol and so obtained a substance five or six times as potent as oestrone. It was afterwards isolated from the liquor folliculi of the ovary, showing it to be a natural production of the animal, by MACCORQUODALE, THAYER, and DOISY (1934). Equilenine and other substances with varying degrees of potency were isolated from urine by GIRARD (1933). Progesterone, an unsaturated diketone, was isolated by ALLEN, W. M. (1932), FELS and SLOTTA (1931), and FEVOLD and HINAW (1932). BUTENANDT (1934) made it also from stigmasterol (a sterol of the soya bean) and established its structure. Androsterone, which is a saturated hydroxyketone, was isolated by BUTENANDT (1931) and has been prepared from cholesterol by RUZICKA (1936). Another male excitant, androstadiol ($C_{19}H_{28}O_2$), has also been prepared by BUTENANDT and by RUZICKA and their co-workers (RUZICKA *et al.*, 1935), but the latter does not occur naturally. The preparation of sex hormones from sterols suggests that in the living animal they may be metabolic derivatives of sterols.

Many synthetic compounds also which resemble oestrin physiologically have been prepared in the laboratory. Some of these bear only a comparatively slight chemical similarity to the true oestrogens, the partially hydrogenated phenanthrene nucleus which they possess being the sole character in common. DODDS and LAWSON (1936), however, quite recently, have reported that they have now produced synthetic agents without the phenanthrene condensed ring-structure, which, therefore, is not necessary for their activity. The natural sources from which oestrus-producing substances have been obtained include a great many animal and vegetable organisms in which they are formed and generally, at least, in the organs associated with reproduction.

In mammals oestrus-producing substances of different chemical composition, besides being obtained from the ovaries, have been extracted from the placenta, the blood, and (in women, mares, sows, and cows) from the urine, more particularly from the urine of pregnancy, but, as demonstrated by ZONDEK (1934), the richest known source is the urine of the stallion. It is to be noted that oestrogens are not normally obtainable from any source in the body after the extirpation of the gonads excepting from animals with retained placenta. After the placenta is removed none of these substances is to be found. Similarly, the formation of the male hormones is apparently confined to the testes, but, as in the female, there are several naturally produced substances which can be made to cause the sexual phenomena. In view of all these facts it is apparent, as DODDS has pointed out (1935, A, B), that the hormone-producing mechanisms are not very specific in their demands for a chemical excitant, and it is all the more remarkable that the body should, so to speak, go to such trouble to produce the particular active substances which are believed to be responsible for the respective sexual processes of the male and female. At present the most reasonable way of regarding the matter is to suppose that the sexual hormones were originally derivatives from the sterols which are widely distributed in living tissues, that the hormones at first had no particular physiological significance, but that in the course of evolutionary progress the parts of the body concerned, the uterus and the vagina and the male accessory sexual glands as well as the secondary sexual structures, have developed the capacity to respond to the chemical substances which have thus acquired the character of specific hormones in the manner originally postulated by STARLING (1935). The mechanism in this respect is, in fact, comparable to that of the regulation of the respiratory movements through the centres in the brain responding to the increased tension in the blood of that simplest and most primitive of all products of metabolism, carbon dioxide. It is interesting to note, further, that the oestrogenic substances which are got rid of in such large quantities in the urine of pregnancy are nearly all in a "combined" form, and possess a very low physiological potency, and that one of these inactive substances has now been isolated and identified by COHEN and MARRIAN.* This discovery is in agreement with the suggestion that the oestrus-producing substances were primitively of no special physiological significance, and it would seem that after the development of the mechanism involving their

* As being probably oestriol monoglucosonide (COHEN, MARRIAN, and WALTON, 1935).

use it may be necessary for the organism at certain seasons to take steps, so to speak, to put them out of action. Nevertheless, the oestrins found during pregnancy, though they do not cause oestrus in the animals producing them, may have a functional action on the uterus, both on the mucosa and on the contractility of the muscle as experiments by ROBSON (1934) and others have shown.

We have seen, then, that oestrus and the corresponding condition in the male are brought about in the individual by the action of chemical substances produced by the gonads. In the female mammal the oestrous processes are followed by a phase due to the hormone formed by the corpus luteum and in pregnancy possibly also by the placenta. In the polyoestrous animal these hormones act alternately until the breeding season is over, the organism then going into a state of sexual rest, but in certain species such as man there is ordinarily no period of quiescence. It is interesting to find that according to FRANK (FRANK and GOLDBERGER, 1928) the phase of the menstrual cycle at which there is the greatest concentration of oestrins in the blood in man is just before menstruation.* This is in general agreement with the view that menstruation corresponds to the end of pseudo-pregnancy (or pregnancy), and in this connexion it is to be noted that ROBSON and HENDERSON (1936) by injecting oestrone or oestriol into a bitch at about this phase (and for some time before) have brought about a uterine condition which resembles histologically that of the menstruating human uterus. In most animals, both in the male and in the female, in the non-breeding season the production of the hormones is much diminished, and this is probably most marked in those vertebrates where the gonads are spent and reduced to a minimum after the discharge of the reproductive products.

THE ANTERIOR PITUITARY

I must now retrace my steps and consider briefly the part played by the anterior lobe of the pituitary. HEAPE (1905), some thirty years ago, put forward the theory that some substance is formed in the body in small amounts that is responsible for both growth and reproduction, pointing out that, broadly speaking, reproduction begins when growth ceases or at least slows down. This hypothetical substance he called tentatively the "generative ferment". HAMMOND (1925) adopted the same idea and used it to explain certain phenomena which had been produced experimentally. For instance, FOA (1901) found that immature ovaries when grafted into a previously oöphorectomized adult underwent a rapid development in the mature somatic environment. Conversely, it had been shown that adult ovaries when transplanted into young immature females lost their adult histological characters and had no perceptible endocrine activity. The mechanism of compensatory hypertrophy of the ovary was similarly explained, as well as the fact that a third engrafted ovary does not add to the number of mature follicles in the animal, the three ovaries together

* According to ZUCKERMAN (1936, A), the actual menstrual discharge is associated with a fall in the oestrin content, but there is a lag in the effect of 7 to 14 days represented by the period between ovulation and the beginning of the discharge. This succession of phases, he claims, can be imitated experimentally by injection of oestrin into oöphorectomized monkeys.

producing about the same number of follicles as is ordinarily produced by two (*see* LIPSCHÜTZ, 1927). On this view of a somatic control of the ovary it was supposed that the problematic generative substance was limited in amount, and limited also in its effect upon the gonad. Subsequently HEAPE withdrew the idea of the substance being a ferment and tended to identify it with vitamin E, while HAMMOND identified it with an active principle in the anterior pituitary gland. Bearing on the same point are the experimental results of PARKES (1929), who found that the ablation of the ovarian follicles in the young mouse before puberty or even in the embryo in the uterus, while it completely eliminated the cyclical histological changes in the ovary after maturity, did not destroy the other manifestations of the oestrous cycle. These experiments suggested that the cycle in the mouse, although dependent upon the presence of the ovary, is regulated by some other factor or factors external to it.

That there was a functional correlation between the anterior pituitary and the sex organs had long been known to clinicians, and sterility and various abnormal conditions and aberrations on the part of the sex organs have been found associated with hyper- and hypo-pituitarism, as described by FRÖHLICH (1901), CUSHING (1932), and many other investigators. In recent years the functional correlation between the anterior pituitary and the gonads has been definitely established, and it has been shown that in the absence of the anterior pituitary the gonads in the young fail to develop and in the adult undergo atrophy. The first experiments dealing with the problematical hormones were those of EVANS (EVANS and LONG, 1922), who showed that simple saline, and more particularly NaOH extracts of ox pituitary, caused the ovarian follicles of rats to become converted into luteal tissue. Shortly afterwards two groups of workers, ZONDEK and ASCHHEIM (1927) in Germany and SMITH and ENGLE (1927) in U.S.A., obtained striking results from the implantation of pieces of anterior lobe tissue into young rats and mice, the ovaries of these animals undergoing marked development followed by ovulation and the formation of luteal tissue and the corresponding oestrous and post-oestrous changes in the accessory organs.

It has been supposed that there are at least two gonad-stimulating hormones produced by the anterior pituitary, since, speaking generally, alkaline extracts have produced marked lutealization and the implantation of pituitary tissue has brought about oestrous conditions. The precise converse of these results has, however, also occurred, so that it is still uncertain whether there are really two anterior pituitary hormones controlling the two main stages of the cycle or only one reproductive hormone formed by the pituitary. Indeed, it is held by some that there is one principal gonadotropic hormone formed by the pituitary, which is probably produced in varying quantity depending upon the external environment and the stimuli derived therefrom, but the question is still an open one.*

* BELLERBY (1933), basing his conclusions on the results of different methods of extraction, whether by acid or by alkali, is of opinion that there is probably only one anterior pituitary gonad-stimulating principle. Thus ovulation in *Xenopus* could be induced by the hormone obtained in both these ways. The anterior pituitary principle which causes ovulation in the rabbit, referred to below, is probably the same hormone.

Moreover, the condition of pregnancy (and also probably of pseudo-pregnancy) must be supposed to react upon the anterior pituitary, and in the absence of such a condition the mammary glands are not built up in the natural state, though they can be experimentally by the injection of oestrin followed by anterior pituitary extract, even in castrates. It has been commonly supposed that the basophil cells of the anterior pituitary are the source of the gonadatropic follicle-producing hormone (and SEVERINGHAUS (1934) considers it to be definitely proved), though with some there is still doubt about this matter (*cf.* LANGDON-BROWN, 1935). WOLFE, PHELPS, and CLEVELAND (1934) have described a cyclic rhythm in the predominant cell type at different stages of the oestrous cycle in the rat, the basophil cells being more numerous at oestrus and the acidophil cells during pregnancy and pseudo-pregnancy. Castration in both sexes has a marked effect upon the anterior pituitary, large, clear cells called castration cells making their appearance, and in correlation with this there is said to be an increase in the gonadatropic hormone content of the gland which may be inhibited by the injection of oestrin (HOHLWEG and DOHRN, 1931); furthermore, with two mice grafted together so as to be in circulatory union, one being a castrate and the other a female, it has been found that the female is maintained in a state of continuous oestrus through the hyperactivity of the pituitary of the castrate (MARTINS, 1931).^{*} For such reasons it has been suggested that anterior pituitary activation is periodically arrested when greater amounts of oestrus-producing hormones are secreted into the blood until such time as the diminution of the gonadatropic hormone reduces the secretory activity of the ovaries (MOORE, 1935). In this way the ovario-pituitary rhythm is supposed to be regulated; that is to say, when the level of oestrus-producing substances falls the pituitary responds with an increase of gonadatropic follicle-producing hormone, and when the level rises the pituitary output is reduced. This explanation, however, if it be true, can only apply to the short or dioestrous cycle in polyoestrous animals such as the rat. It cannot apply to the alternation between the sexual and non-sexual or anoestrous seasons, for during the anoestrus the whole reproductive system goes into a state of prolonged rest which should perhaps be regarded as a result of exhaustion consequent upon activity. This is seen also in birds in which the pituitary makes no attempt at any time to maintain an adjustment, the ebb and flow of follicle-stimulating and ovarian hormones being approximately coincident.

In the bird, however, as described by WIRSCHI (1935), the reactions to the various gonadatropic substances are not quite the same as in mammals. The follicle-stimulating substances appear to be far more potent in their effects upon the bird's gonads than those which in the mammal tend to cause lutealization. WIRSCHI found that in the finches on which he worked, the most perfect results were obtained from the injection of pregnant mare's serum, which contains an anterior pituitary-like principle, the hens ovulating and laying eggs. This happened even in cases where

^{*} For further information and references, see article by SEVERINGHAUS, ENGLE, and SMITH in ALLEN's "Sex and Internal Secretions" (1932), also MOORE and PRICE (1932), SEVERINGHAUS (1934), and MOORE (1935).

the birds otherwise never lay in captivity (in the African weaver birds), while in the males there was full spermatogenesis.

WALTON and I (unpublished) found that by injecting an anterior pituitary principle made from human placenta into female white-fronted geese (a species which does not breed in this country, not even in captivity, or only very seldom) the ovaries could be induced to grow and produce good-sized follicles, but the birds did not lay, possibly because the surroundings were not sufficiently suitable.

Gonad-stimulating substances (anterior pituitary-like principles) have been obtained from human urine, especially during pregnancy, which correspond physiologically more or less to the two anterior pituitary principles, as well as in large quantities from the placenta (ZONDEK and ASCHHEIM, 1928, ZONDEK, 1931), and from blood serum (as already indicated), but their physiological action, although similar, is not identical (COLLIP, 1932, CAMERON, 1935). Moreover, substances have been extracted (BELLERBY, 1929, FRIEDMAN, 1929), both from the pituitary and from the urine which cause ovulation, and others have been obtained from the pituitary which appear to act directly on the mammary gland, promoting growth and also secretion (CORNER, 1930, ASDELL, 1932, RIDDLE, 1935). Extracts of urine of pregnancy (and sometimes anterior lobe extracts) often give rise to haemorrhagic follicles in the ovary and these are unruptured; sometimes also the follicles may become lutealized without the ovum being discharged.

The chemical composition has not been determined in the case of any of these anterior pituitary substances, and in no instance have they been obtained in crystalline form. In the absence of further evidence it seems probable that many of these substances, which have slightly varying biological properties, are products of metabolism or chemical derivatives of one or two gonad-stimulating hormones in just the same kind of way as there is a number of oestrus-producing substances that are probably derived from the principal hormone of the ovary, or certain of the anterior pituitary-like principles may be formed in the placenta from which they have been obtained. As shown by CATCHPOLE, COLE, and PEARSON (1935), whereas some of these substances are excreted in the urine there are others which after circulating in the blood are destroyed somewhere within the body.

Here it may be mentioned also that RIDDLE has extracted from the anterior lobe a substance (which he considers to be a distinct hormone) which produces full development of the crop-gland in the pigeon at any time in the cycle and in both male and female and also in castrated birds (RIDDLE and BRAUCHER, 1931). It is obtained from a definite fraction and does not produce the effects which characterize the growth and gonadotropic hormones of the pituitary. This substance, which he calls prolactin, also causes the incubation instinct in fowls and the associated regression of the ovary, besides producing active lactation in mammals (provided that the mammary glands are already fully developed) and exciting the various maternal instincts associated with the feeding and care of the young (RIDDLE, 1935). It appears to be a different substance from that which may stimulate mammary growth, as described by (CORNER 1930). Here we have a definite example of a

substance which presumably existed as a product of metabolism before it became a hormone and to which various other organs—the crop gland, the ovaries, and the mammary glands, came to respond in the progress of evolutionary life. Anyway, it existed before the main vertebrate stem divided into birds and mammals, though in some of the more primitive forms it may already have possessed a function. As we have seen in the pigeon, it appears to control a certain stage in the cycle. In the rat the release of prolactin at parturition may result from afferent stimuli occurring during that process.

We have now seen that the gonads through their respective hormones act upon the accessory organs and other tissues concerned in the sexual cycle, and that the anterior pituitary gland acts similarly by its hormone or hormones upon the gonads. We have seen, further, that in the female there may be secondary or dioestrous cycles within the major cycle and that these also are dependent upon the ovarian hormones which are successively produced. Further, the duration of the corpus luteum depends partly, at any rate, on the anterior pituitary, as shown especially in such species as the rat. As to whether the ovarian hormones react upon the anterior pituitary at certain stages in the cycle we have no completely satisfactory proof, but it is almost certain that such an influence exists. The castration effect must be regarded as definitely proved.

As to whether the suprarenal and the thyroid exert any effect on the oestrous cycle in any way other than that they may be supposed to condition it, there is no clear evidence. The facts have been well presented by SMITH (1932), who supplies many references. DEANESLY (1928) has shown that in the rat and mouse a high percentage of animals survive after adrenalectomy and that the cycles are normal or slightly lengthened. Most other recent investigators have got similar results. The evidence as to the thyroid is also unsatisfactory. According to BENNETT (1934), however, this gland plays a part in determining the periodic testicular growth in the drake. The conclusions are based on the results of injections of thyroid hormone as well as on the effects of removal of the thyroid.

We may now consider the evidence as to the existence of the exteroceptive or other stimuli which regulate the gonadotropic activities of the anterior pituitary.

EFFECTS OF LIGHT AND ULTRA-VIOLET IRRADIATION

The first to show experimentally that light was a cause of cyclical reproductive activity was ROWAN (1926, 1930), who conducted an investigation upon the migratory junco finch of America. By exposing the birds in mid-winter in Canada to ordinary electric light he obtained an increase in the size of the gonads comparable to what occurs with the increase of daylight in spring. Warmth was clearly not a factor in the process since the birds were kept at the temperature of the outside environment which was very cold. The results were confirmed with crows and canaries, and BISSONETTE (1933) extended them to the European starling, for which he

found that intensity and wave-length, as well as daily periods of light, are factors in promoting sexual activity, while the method of increase of light also played a part. Moreover, **BISSETTE** (1932), as a result of researches in Cambridge, made the important discovery that with increased illumination by electric light (200-watt bulbs) in mid-winter and therefore at the time of the normal anoestrus ferrets came into full oestrus with typical vulval swelling in 38 to 64 days. In the male ferret the response was less complete, for although the accessory organs developed and mating took place, owing to the incompleteness of spermatogenesis the matings were followed by sterility. At the same time, **BAKER** and **RANSON** (1932), at Oxford, showed that the oestrous cycle in the vole could be modified by varying the rations of artificial light though food and possibly other factors might alter the response. Furthermore, the effect of illumination with ordinary electric light in producing oestrus in the ferret was fully confirmed by **HILL** and **PARKES** (1933). These investigators showed also that the anterior pituitary is bound up with the sexual photoperiodicity since hypophysectomized animals are unaffected by artificial lighting and go into permanent anoestrus unless injected with pituitary extracts. This is in conformity with what we know about the function of the pituitary in other animals and with the experimental results of pituitary implants and the injection of extracts.

The question as to whether the acceleration of the recurrence of the cycle is brought about by general light and heat radiation or whether the effect is due to stimulation by definite portions of the spectrum was then investigated by **BOWDEN** and myself (**MARSHALL** and **BOWDEN**, 1934). It was found that for the ferret heat rays and the near infra-red were inactive. The effect begins with the red radiation and extends throughout the visible to the near ultra-violet. Pairs of ferrets were subjected, further, to the same total quantity of radiation from incandescent lamps, but in one case it was concentrated into two hours, and in the other spread over 16 hours. The results were similar in each. The ferrets which were subjected to ultra-violet remained on heat for a longer time than the others, although the irradiation had for some months been discontinued; that is, they remained on heat for five or six months after irradiation ceased. The following year the results were similar, and this year they are so far similar again.* Further, ferrets which were subjected to incomplete darkness did not come on heat, but individuals which had already begun to come on heat entered into full oestrus and remained in that state for a normal period. A blind ferret (with cataract) did not come on heat at all, although kept under observation for two years, during which time it was apparently healthy and fed well. **HILL** and **PARKES**, however, found that in ferrets subjected to darkness there

* The two ferrets subjected in the spring to ultra-violet irradiation have been continuously on heat since February, while those brought on heat by other rays "went off" after several weeks, but are now (after the manner of normal ferrets) on heat a second time (20 July, 1936). The two ferrets irradiated with ultra-violet light finally "went off" heat at the end of the summer, behaving like those similarly treated in previous years, and in contrast to those treated with light which "went off" much earlier (6 October, 1936).

was on the average some lag in the times of onset of oestrus but this was slight. **BISSETTE** (1935), on the other hand, found that by employing hoods or curtains or otherwise reducing the light received there was a definite arrest of the cycle, that with ferrets already on heat treatment by darkness led to regression, and that in general the onset and duration of oestrus could be regulated artificially by changes in the duration or intensity of the light to which the ferrets were exposed. Further, **BISSETTE** (1936) found that severing the optic nerves of ferrets frees the cycle from the influence of the seasons and sexual activity can occur at abnormal times (June to November or July to December instead of March to August). In all the experiments on ferrets warmth may be ruled out as a factor in the periodicity—the controls as well as the results of infra-red radiation show this—while food may also be excluded since the irradiated ferrets ate no more than the normal animals.

- It seems probable that the great fecundity of the marmoset colony at the Lister Institute, where these animals were induced to breed after irradiation with ultra-violet light was not merely the result of improved health due to the anti-rachitic effect of the treatment, but that the rays may have had a stimulating influence also on the anterior pituitary, promoting the secretion of the gonadotropic hormone. Miss **MARGARET HUME**, to whom I am indebted for unpublished information, states that some of the marmosets produced an excessive number of foetuses which in certain cases could not be born and that even the mother of the colony started having triplets instead of the normal two. These results of ultra-violet treatment are probably comparable to those associated with super-ovulation in mice when injected with anterior-pituitary extracts.

In the meantime, **KIRSCHBAUM**'s (1933) experiments on the sparrow showed that artificial lengthening of the day brings about a precocious development of the sex glands, especially in the male in winter, and **WITSCHI** (1935) repeating the experiments obtained the same effects. Moreover, **COLE**'s (1933) researches with the mourning dove of America and experiments upon mejjoros, turkeys, guinea fowl, pheasants, quail, and grouse, conducted on the same lines, led to similar results (*see* **BISSETTE**, 1936). In the experiments upon ducks by **WALTON** and myself, artificial lighting in the spring accelerated the cyclical change, as was shown by the birds displaying courtship phenomena, the drakes fighting, and the occurrence of the seasonal eclipse in April instead of at the usual time in July. But in these experiments, probably as a result of otherwise unsuitable conditions, the birds did not lay. **BENORR** also has shown that light has a pronounced effect in stimulating the testis of the drake, both immature and mature birds being affected. When a hood was used to cover the eyes the effects did not supervene. **BENORR** (1934), therefore, at first concluded that the stimulus must pass through the eyes and optic nerves and brain and thence to the anterior pituitary, but in his later experiments, just published (**BENORR**, 1935), he severed the optic nerves or removed the eyeballs. Under these conditions the effect of lighting still led to acceleration, yet hooding of the eyes and the region around them had an inhibitory effect. **BENORR** concludes that there must be some other receptor organs in this region, but the matter remains obscure.

According to the recent observations on sparrows by IVANOVA (1935), the plucking of the feathers of irradiated birds resulted in a greater augmentation of testicular growth than in birds with feathers, but the latter also underwent an increase. There is probably considerable species variation, and though the eyes normally receive stimuli which are transmitted through nervous paths to the anterior pituitary, the effects in some animals are conveyed by alternative paths. In this connexion, HOGBEN's observations on the result of irradiation on the pituitary of the Cape clawed toad are not irrelevant, although in this case the effect was shown in another function of the gland. HOGBEN found that in *Xenopus* the secretion of the melanophore hormones of the pituitary is stimulated through colour vision. "In the eyeless animal, the melanophores are neither fully expanded nor fully contracted. The dark background response (fully expanded melanophores) is caused by photoreceptors in the fundus of the eye which reflexly stimulate the pars intermedia (of the pituitary). These receptors are most sensitive to light from the red end of the spectrum and are hardly affected by blue-green rays. On the other hand, the photoreceptors of the white background response are located in the periphery of the retina; they are not sensitive to red light and appear to control the activity of the pars tuberalis. On this view, the two responses are separate entities and the secretion of two different hormones of the pituitary is differentially controlled by different wave-lengths" (HOGBEN, private communication to Miss WHETHAM, WHETHAM, 1933). These results are interesting as showing that in some animals at any rate the activity of the pituitary gland is directly affected by irradiation through the retina. Moreover, it was found that in blinded toads (*Xenopus*) the ovary was under-developed (HOGBEN, CHARLES, and SLOME, 1931).

It may again be remarked that increases in light have no effect if the pituitary is removed (as shown by HILL and PARKES for the ferret), and further, that the same effects as those produced by light can be evoked at any season of the year by the injection of the gonad-stimulating principles.* Again, BISSONETTE (1936) has shown that the pituitaries of stimulated ferrets undergo histological changes similar to those of castrated animals, large clear cells being produced. The effects of stimulation, however, are not permanent, for the animals eventually go into a state of sexual rest in spite of the continuance of the stimulating agents used.

It must, of course, be freely admitted that all animals do not respond to an increase of light; there are exceptions both among mammals and among birds. YOUNG and his collaborators found that the cycles in guinea-pigs are but little affected by changes in light or by darkness (see DEMPSEY and others, 1934), and MOORE and his collaborators (MOORE, SIMMONS, and others, 1934), adopting similar methods, did not obtain any alterations in the sexual cycles of the spermophile. That there is variation in passing from species to species is shown also in birds, for whereas the Adélie penguin in the Antarctic breeds in the warmest and lightest time of the year the Emperor penguin lays its eggs in the dark. In some species of mammals the

* The possibility must always be borne in mind, however, that light may sometimes act by producing chemical changes in the skin and not through receptor organs.

gonads begin their annual development in winter before the days begin to lengthen, and in birds which migrate from the southern hemisphere to the northern the periodic enlargement commences before the migration starts, and in crossing the Equator the birds pass to countries where the days are shorter. In the African weaver finch, to take an instance, the breeding season is in the autumn. These birds must presumably react to diminution and not to increase in light. WIRSCHI (1935) records that he has kept these birds at Iowa under constant food conditions and that their autumn breeding seasons are most regular; also that young birds without exception fell into line with their adult companions, even though they had travelled widely in different parts of the country. With tropical birds living where there is little or no variation in the length of day, seasonal breeding may still occur, as recorded by BANNERMAN (1930, 1931, 1933) and others, and it is suggested that it is determined by the recurrence of the rains and various ecological factors. These factors may also act through the intermediation of the nervous system upon the anterior pituitary.

The outstanding fact remains, however, that in nearly all animals breeding phenomena occur in response to seasonal change, and in the vast majority of these (but not in all), as shown by observations under both natural and experimental conditions, the principal stimulus is increase of light. Changes in temperature and food are generally eliminated by control observations, though in some cases these factors not merely condition the phenomena but play a part in determining the sexual cycle.

The general conclusion is reinforced by statistical studies of the times of breeding of the domestic animals. In view of the practice adopted by poultry keepers of using artificial light to increase and extend the time of egg production, Miss WHETHAM undertook a statistical investigation based on available records to ascertain if there were any relation between egg production and variation in daylight in different latitudes, and found that there was a correspondence which though not absolute was nevertheless definite. Similarly, in the case of horses, although in the more domestic breeds (*i.e.*, in the "better bred" or more improved types) the dioestrous cycle may recur for a great part of the year, there is a definite tendency for foaling to occur in the spring. Dr. HAMMOND, to whom I am indebted for the information (at present unpublished) based on his statistical researches upon the records obtained from the stud books of different countries, has constructed frequency curves with peaks showing when foaling was most common. In Canada, as with Britain, there is a very pronounced peak in May. In the United States, which extends far down towards the Tropics, where the seasonal differences are less, there is for the whole country a definite peak also in May but not so pronounced as in Canada. In Australia and New Zealand there are very marked peaks in October and November respectively, in the spring of these countries. In South Africa there are only slight peaks in September and again in November. In India, where comparatively speaking conditions are much more uniform, there is only a slight peak in March. The gestation period being eleven months, the service peaks are in all cases a month later than the foaling peaks. The results as a whole undoubtedly suggest a correlation

between the sexual season and the incidence of daylight. HAMMOND noted, further, that with the Shetland pony, which is comparatively primitive, the service and foaling peaks were more pronounced than with the more improved breeds. It may be recorded, further, that COSSAR EWART imported a pony from the Island of Timor, which is in the southern hemisphere, into Scotland and found that whereas in the first year it came on heat in the autumn, which synchronizes with spring in Timor, it afterwards adjusted itself and underwent recurrent oestrus in the spring in Scotland.

The usual sexual season for sheep, especially for the hill breeds, is autumn, as also is the case with deer, these animals being among the exceptional species which appear to respond to diminution rather than to increase in daylight. In the southern hemisphere their breeding season is in actual time the exact reverse of what it is in Europe. But what is more interesting is that Dr. ROUX (1936), to whom I am indebted for supplying me with unpublished information, found that individual sheep on being transported from Britain to South Africa adjusted themselves to the reversed seasons, coming into line with others that were born and bred in South Africa, thus showing that the major cycle is definitely determined in sheep by external stimuli rather than by an inherent reproductive rhythm. The behaviour of these animals was clearly comparable to that of EWART's Timor pony. ROUX states, further, that the sheep were fed uniformly and consequently nutrition could not have been a factor in the rhythm.* (See Postscript.)

OTHER EXTEROCEPTIVE FACTORS

I pass on now to consider the evidence as to the part played by exteroceptive stimuli arising from the relations between the sexes and the relations between the mother and her offspring in controlling or modifying the phases of the sexual cycle.

* I am indebted to the Marquess of TAVISTOCK for kindly supplying me with the following important information about the breeding habits of foreign birds in captivity :—"Tropical birds, such as doves and finches, that breed for the greater part of the year in their own country often attempt to do so in this, usually with fatal results to the hens and young. In some cases, however, the cold, short days and restriction of highly-vitamized food will affect their general health adversely to a sufficient degree to discourage breeding.

A few species succeed in rearing their young successfully even during our winter—the lovebirds, *Agapornis personata*, *fischeri*, *liliana*, and *nigrigenis* being noteworthy examples. Some Australian lorikeets (*Trichoglossus*) will also breed successfully in mid-winter, as will the budgerigar, zebra finch, etc.

The emu shows little willingness to adapt itself to our seasons, usually laying in mid-winter. Only exceptionally do pairs defer breeding until the spring.

The rheas, however, always adapt themselves to our seasons and, as a rule the ostrich does likewise. Birds from North Australia are markedly more inclined to stick obstinately to their own breeding season (October). The hooded parrakeet (*Psephotellus cucullatus*) and BROWN's parrakeet (*Platycercus venustus*) are a case in point. Only after some years and a good deal of encouragement (by the removal of nests in autumn and their return in spring) can some pairs be induced to adopt our seasons and their English-bred offspring are as inclined to spring-moulting and autumn-breeding as their parents even when spring-bred themselves. BROWN's parrakeets often have two moults in this country; one in May when they would naturally moult in Australia; and another in August when they would moult if they had adopted our seasons."

The best-known examples of this kind of phenomenon are probably those supplied by the rabbit and the ferret, and also by the ground squirrel, and under certain circumstances by other animals which normally only ovulate in response to the stimulus set up by coition or by the orgasm. Sterile coition in the rabbit and ferret is followed by pseudo-pregnancy with mammary development and secretion. The stimulus therefore causes a switch over from the oestrous or follicular phase to the luteal phase. Moreover, the switch cannot be effected in the absence of the pituitary, whereas, on the other hand, it can be brought about by the injection of anterior pituitary or anterior pituitary-like extracts (FEE and PARKES, 1929). The presumption is that ovulation is due normally to a nerve reflex but the stimulus apparently may be carried by several nervous paths. Thus, it is not necessarily started from the vagina and vulva, since FEE and PARKES have shown that local anaesthesia of these parts does not inhibit ovulation after coition. Ovulation can likewise occur after complete thoraco-sympathectomy (CANNON and his pupils, 1929), and in the absence of any nerve pathway to the ovaries (HINSEY and MARKEE, 1932); also after ovarian transplantation to an abnormal position (ASDELL, 1926, FRIEDMANN, 1929). It can also occur in the rabbit after cervical sympathectomy (pseudo-pregnancy following) (VOGT, 1933, HATERIUS, 1933). Nerve fibres have been found in the pars intermedia by CROLL (1928) and others, but their source and origin have not been traced. LOEWI (1935), in his Ferrier lecture given to the Royal Society a year ago, remarked that the anterior pituitary appears to receive its messages humorally and transmits them in the same way.

VERNEY and I (1936) have found that the natural method in the oestrous rabbit can be imitated by a strong electrical stimulation of the central nervous system,

Parrakeets from Southern and Central Australia adapt themselves to our seasons at once. If imported in winter they moult soon after arrival and again in August; after that in our late summer and autumn only. In the case of grass parrakeets (*Neophema*) and Bourke's parrakeet, conditions of captivity appear to induce much greater prolificacy and several broods may be reared. In a wild state, these species appear at most only double-brooded and apparently usually single-brooded." (See also TAVISTOCK, 1935.)

Mr. DAVID LACK informs me that the cases of the black swan, the *Cereopsis* goose, and the emu (see above), which have been quoted as examples of birds which retained their southern hemisphere breeding seasons in England, were based on mistaken evidence as to their breeding seasons in Australia. ZUCKERMAN states that many species of mammals which have bred in the London Zoological Gardens (e.g., polar bears, reindeer) have retained their original breeding habits, but none of the animals cited came from the southern hemisphere. On the other hand, according to PYCRAFT (1913), the Chital or spotted deer from India, on being introduced to Europe, was at first in danger of extinction owing to the calves being born at an unsuitable season (in mid-winter) and consequently dying, but the animals afterwards adapted themselves to the new conditions and bred freely at an appropriate time. ZUCKERMAN says on the authority of SCHUSTER that several mammals living in East Africa breed at any time of the year, and also on the authority of STEVENSON-HAMILTON, that the evidence suggests that the lion has no demarcated breeding season. It may be noted further, that the goat in South Africa, like the sheep, has reversed its breeding times to conform to the seasons of the southern hemisphere. (For further information and references see MARSHALL and BOWDEN (1934) and ZUCKERMAN (1932).)

and ovulation followed by pseudo-pregnancy produced. The stimulus was equally effective whether applied through the brain or through the lumbo-sacral part of the cord. In a few animals, however, haemorrhagic follicles were produced instead of corpora lutea, but it is to be observed that this also often happens after injecting anterior pituitary-like principles. Further, ovulation did not supervene until 17 to 24 hours later instead of the usual interval of 10 hours, and pseudo-pregnancy might be a day or two shorter than usual. It is clear, however, that an electrical stimulus could switch the cycle over from the oestrous to the luteal phase. FRIEDGOOD and PINCUS (1935) found that ovulation could supervene after stimulation of the cervical sympathetic ganglia, but, as in our experiments, there was a delay of 12 to 14 hours beyond the normal interval, the result in this respect being in agreement with our observations. FRIEDGOOD and PINCUS's experiments are remarkable in view of the fact recorded above that ovulation can occur in the rabbit after complete cervical sympathectomy. One may, perhaps, interpret the results on the assumption of more than one nervous path and more than one mechanism for the initiation of the processes. HATERIUS (1934), however, could not produce ovulation after cervical sympathetic stimulation.

HARRIS (1936, B) has succeeded in producing ovulation in the rabbit and ferret after local stimulation of the hypothalamus, but it is just possible that the effect was due to an extension of the stimulus to nerve endings in the pituitary itself. There was a long delay—30 to 40 hours, before ovulation occurred, and in some cases instead of ovulation taking place haemorrhagic follicles were formed or luteal tissue without the discharge of the ovum, in just the same way as often happens after the injection of anterior pituitary-like principles.

In the rat, unlike the rabbit, ovulation takes place spontaneously, but a prolongation of the life of the corpora lutea with consequent pseudo-pregnancy can be induced by sterile coition or on artificially stimulating the cervix uteri by mechanical or electrical means (LONG and EVANS, 1922, SHELESNYAK, 1931). MEYER, LEONARD, and HISAW (1929) found that general and spinal anaesthesia inhibited the occurrence of the pseudo-pregnancy which otherwise follows electrical stimulation of the cervix. BACQ and BROUHA (1932) found that sympathectomy had no effect on the cycle in the female rat and they do not mention the occurrence of pseudo-pregnancy. HARRIS (1936, A) found that electrical stimulation through the brain of the oestrous rat, after the manner of VERNEY and myself for the rabbit, caused definite pseudo-pregnancy, and deciduomata could be induced to form in response to a mechanical stimulus introduced in the uterus. The observations show very clearly that the switch over from the oestrous to the pseudo-pregnant condition in the rat must be due to a change in the anterior pituitary and not merely to the corpus luteum which is formed in any case, whether a stimulus is applied or not. SELYE and McKEOWN (1934) have shown that mechanical stimulation of the nipple without the withdrawal of milk (as after the removal of the galactophores) in both rats and mice, also produces a prolongation in the duration of the corpora lutea, but the stimulus must be continued if this effect is to be produced. The well-known fact that suckling is

normally essential for the continuance of lactation in mammals is similarly to be accounted for on the assumption that exteroceptive stimuli are conveyed from the nipples to the anterior pituitary by nervous paths. And the further fact that menstruation in women in about 60% of cases is inhibited by suckling is perhaps also relevant.

General evidence of a functional correlation between the hypothalamus and the gonads is furnished by the described effects of hypothalamic lesions in inducing the symptoms of *dystrophia adiposo-genitalis*. SMITH (1930) has included genital atrophy among the results of injury to the tuber cinereum in the rat, but according to CUSHING (1932) the gonadal effect may be an instance of interference with the hypophyseal blood supply or with its nerve supply. CUSHING, however, after a discussion of the clinical and other evidence, strongly emphasizes the essential unity of the diencephalo-hypophyseal mechanism.

Lastly, THEOBALD (1936), has collected evidence showing that drugs such as morphine as well as psychical factors (suggestion, etc.) may affect the rhythm of the menstrual cycle in women, and he suggests that the results are transmitted through a centre in the hypothalamus, but further evidence is required before such a conclusion can be established.*

Instances have been given above from among mammals showing that the succession of the phases of the cycle in some species may be modified by exteroceptive stimuli acting through the intermediation of the nervous system upon the anterior pituitary gland. Cases of the same kind of phenomena are also very common among birds. There is no luteal phase in birds, but as we have already seen, there occurs from the beginning of the season of reproductive activity a succession of phases which manifest themselves in the bird's behaviour, and which often grade into one another and to a certain extent overlap. There is, of course, much species variation, but speaking generally the order is as follows: Movement to the breeding area which may consist of migration over long distances, this occurring contemporaneously with the periodic development of the gonads, the taking up of territories (where this is done), pairing, sexual display, coition, nest building (during which display and coition are usually repeated), and in the female ovulation and egg laying, and then, sometimes for one and sometimes for both sexes, incubation, and the feeding and rearing of the young. Here I may quote ELIOT HOWARD (1935):—"No one doubts that the generative organs determine sexual activity, though all may not agree that prooestrus and

* Sir FLINDERS PETRIE has suggested to me that the lunar cycle in women may have become "fixed" in primitive people by the custom of promiscuous dancing, etc., at the full moon, the idea being that these influences may have acted exteroceptively (*cf.* ELLIS, HAVELOCK (1900), who notes that various persons have made similar suggestions). It must be mentioned, however (as perhaps against this idea) that some of the lower Primates have a lunar menstrual cycle. In the chimpanzee the cycle is 35 days (ELDER and YERKES, 1936). In the baboon, according to GILLMAN (1935), it varies from 28 to 63 days, the variation being produced partly by seasonal variation and partly by emotional disturbances. Here we have a suggestion of the cycle in a lower primate being modified by exteroceptive factors.

oestrus in a bird depend upon ovarian processes comparable with the uterine changes in a female mammal, or, indeed, that these phases are really there ; what some may doubt is the wider view that behaviour which custom thinks a matter of mind is in some way determined by changes in the body. True, no change in testis or ovary, or hormone in the blood stream, has yet been found to correspond with territory, with building shells, or with care of young. But from point to point, consider the events. In the spring a male shuns his own kind and makes himself public ; singing, if a song bird ; drumming on a dead branch, if a pied woodpecker ; wandering in air like a butterfly or dancing in it like a moth, sparrow-hawk, godwit, curlew, with slow flapping flight, redshank and dunlin with vibrating wings, each after his kind ; moreover, he defies intruders, threatens attacks, fights until one or other yields ; and before a mate finds him he builds a nest, if a whitethroat ; or a platform, if a waterhen, and broods upon it ; or, like a lapwing, bores hollows in the ground to enjoy it, or merely carries stuff in its beak to drop it anywhere." In each species there is a characteristic succession of phases in both sexes, and this, as HOWARD supposes, must be related to internal change. The study of bird behaviour has already become a large subject, and following upon HUDSON (1919), SELOUS (1928), JULIAN HUXLEY (1914), ELIOT HOWARD (1929), and others, as a result of the intensive watching of birds in a state of nature have made important observations upon the relations of the sexes. But concerning the physiological causes which are responsible for the successive phases we have little knowledge beyond those endocrine factors which I have already recorded. Here I confine myself to some further matters where there is direct evidence as to the effects of exteroceptive stimuli in altering the succession of the physiological processes which constitute the cycle.

The first point I wish to refer to is one which is well known to all ornithologists. For any one species the number of eggs in the clutch is generally constant within narrow limits, that is to say, there is a tendency to lay a definite number of eggs and then to brood over them. If, however, the eggs are withdrawn shortly after being laid, many birds will go on laying, making an attempt, so to speak, to lay up to the right number, and the incubation phase in the cycle is postponed in correlation with the repeated ovulation. One of the best-known examples of this is the case of a wryneck, a species that normally lays 7 or 8 eggs and which, in this instance, laid 42 eggs as a result of the daily withdrawal of the egg deposited. The incident was repeated in the next year when the wryneck again laid 42 eggs, before its ovary was exhausted (YARRELL (1882), KIRKMAN (1911-13)). Similarly, it is recorded that a swallow, by having one of her eggs taken from her daily, was induced to lay 19 eggs (RAY 1848). JESSE (1842), mentions similar observations upon the blackbird, the lark, and the long-tailed tit. About the lark he says, "if only one or two eggs are allowed to remain in the nest, the bird will go on to lay for a time indefinite, but if there are three she will sit. The usual number of eggs in a lark's nest is five." WITSCHI (1935) found that for the house sparrow whereas the normal number of eggs is four or five in a clutch, by removing the eggs daily, the bird may lay up to 50 eggs in succession and often 12 to 19 on consecutive days. On the other hand, if the full clutch is

allowed to remain, the ovary rapidly regresses during incubation through degeneration of the larger eggs, a result which occurs, according to RIDDLE, through the secretion of the anterior pituitary hormone prolactin. In the case of the common fowl, as is well known, as a result of domestication and prolonged artificial selection, the maternal habit of broodiness may be bred out, while the bird has become a veritable factory of egg production. Since in the fowl, and therefore presumably in most birds, there is an interval of only about a day between ovulation and the laying of the egg, it follows that in the cases just cited the repetition of ovulation must be the result of exteroceptive stimuli, though whether the stimulus is derived from perception by the eye, or through tactile perception by the ventral surface of the body, is uncertain. In any case, presumably, the stimulus must pass through the intermediation of the nervous system to the pituitary and so interfere with the normal course of succession in the sexual cycle, the incubation phase being postponed for a long period or indefinitely. Moreover, if the nest of the bird is removed along with the clutch, the nest building may be repeated and the succeeding egg-laying and incubation periods are deferred accordingly, ovulation being resumed in due course. The succession of the phases, therefore, is not merely a matter of cyclical endocrine control (CHANCE, 1936).*

So far we have been considering the exteroceptive factors responsible for the continuance of ovulation after the process has once started. The question as to the initiation of the first seasonal ovulation which I have reserved for longer consideration is another matter. Like mammals, birds in respect of ovulation fall into two categories, (1) those that ovulate spontaneously when the ovaries are in the appropriate condition, and (2) those that require an additional stimulus which is usually provided by the male. The common fowl and the pheasant are examples of the first kind; the pigeon is an example of the second. Concerning the pigeon, HARPER, in a paper published in 1904, writes as follows: "When a pair ready for mating is put together, egg-laying ordinarily ensues at the end of a rather definite period, at the least eight days. The female functions are held in abeyance till the proper stimulus is received from the mate. The maturing of the egg is so exclusively a female function that it seems odd at first thought that an apparent exception should occur to the rule. Of course, we know that the final maturation of the egg, or the giving off of the polar bodies, awaits in most animals the act of fertilization. But here the effect is produced upon the egg by the entrance of sperms. How mating and the act of copulation (which is repeated at frequent intervals every day at this time) could influence the ripening of the egg in the ovary is another problem. In this connexion the curious fact must be mentioned that two female pigeons placed in confinement may both take to laying eggs. The function of ovulation is in a state of tension, so to speak, that requires only a slight stimulus, 'mental,' apparently in

* By removing the complete clutch, CHANCE obtained "repeat layings" in the blackcap, carrion crow, raven, dunlin, and golden plover. Further, the house sparrow, garden warbler, kestrel, and merlin could be induced to lay three complete clutches, and the red-backed shrike four clutches, but there was some reduction in the normal number of eggs in the fourth clutch.

this case, to set the mechanism to working. At any rate, it is impossible to regard the presence of sperm in the oviduct as an essential element of the stimulus to ovulation, although it may have an important influence in the normal case. Our attention is directed to the various and complex instincts of the male which come under the head of courtship, both before and after mating is effected, as furnishing a part of the stimulus to the female reproductive organs." HARPER proceeds to describe a curious habit which is common among pigeons before copulating. The male bird regurgitates some secretion in its throat, presumably from the crop gland, and this is taken up by the bill of the female in much the same manner as the young take their food. "It is easy to see that here may be one of the sources of indirect stimulation to the female reproductive organs." Numerous observations on the pigeon have been made likewise by WHITMAN (1919), who speaks of posturing as self-stimulating. Confirmatory observations have also been made recently by MARTINS (1935). That the display is accompanied by a high emotional disturbance in all birds that show it is very apparent.

The Marquess of TAVISTOCK, to whom I am much indebted for giving me the benefit of his extensive experience of birds of all kinds in a state of captivity, states that apart from gallinaceous birds he has very seldom known egg-laying to occur in any species excepting in the presence of the male. Spontaneous ovulation, in his experience, is non-existent or most rare in species which normally mate for life. It may occur, however, in unmated swans and with various kinds of cranes when two females live in abnormally close association. Otherwise, Lord TAVISTOCK informs me, these birds never lay unless mated. The conditions are clearly comparable to those seen in the pigeon. The mere presence of another individual provides an exteroceptive stimulus which acts on the pituitary and starts the nest-building and ovulating stage of the cycle.

THE MEANING OF SEXUAL DISPLAY

In view of such facts as these it is easy to see that sexual display and courtship phenomena generally probably serve an important function in producing the necessary synchronization of the male and female reproductive processes without which procreation cannot be accomplished. Such a view has been put forward by ELIOT HOWARD, and adopted by PYCRAFT (1913), and also by HUXLEY (1914), who has studied sexual posturing in the great crested grebe. HOWARD says that the purpose of posturing is the provocation of sexual reaction by mutual stimulation, HUXLEY refers to it as having an aphrodisiac action, and BEEBE (1931), supposes that it has "a slow indirect effect upon the nerves". Here I suggest for it a more precise physiological signification.

It has been shown that the gonad-stimulating hormone of the pituitary will cause ovarian development and ovulation in birds, and that sexual posturing or even the mere association of two individuals will initiate nest building and ovulation. There is a presumption, therefore, that sexual posturing produces exteroceptive stimuli which act upon the anterior pituitary through the hypothalamus, and so effects the

necessary synchronization between the sexual processes of the male and female birds. Herein then, in all probability, lies the biological or race-survival value of sexual display and of the adornment which in many species is taken advantage of to render the display the more effective. Those birds which have brighter colours, more elaborate ornamentation, and a greater power of display must be supposed to possess a superior capacity for effecting by pituitary stimulation a close degree of physiological adjustment between the two sexes so as to bring about ovulation and the related processes at the most appropriate times. Upon the basis of this theory we may, if we like, construct an hypothesis as to the evolutionary development of the display and the acquirement of adornment and of the aesthetic sense, comparable to Darwin's theory of sexual selection, and without encountering the main objections to which that theory is open. DARWIN (1871) applied his theory generally to all cases of sifting in relation to pairing and more especially to those involving the preferential but not necessarily conscious choice by the female of that particular male which by his superior beauty and more effective posturing was most attractive to her. Moreover, DARWIN made use of the argument that unless the female is influenced by the male so as to select him, the display of the males before her is meaningless. In the light of the theory of mutual stimulation postulated here, the display is not meaningless at all but subserves a definite purpose. According to this theory, it is not the female which selects the male; it is the pair which have the highest capacity for mutual stimulation which are, so to speak, selected by Nature for the perpetuation of the race. Nevertheless, that sexual selection occurs in some species, such as the ruff and the blackcock, would seem probable in view of the observations of SELOUS (1928), and it also appears to take place on occasion with various species of ducks. Yet as a generalization of wide application the theory fails. WALLACE (1889) on various grounds, rejected it, and MORGAN (1903) advanced no fewer than twenty reasons as to why it cannot be true. Some of the objections are supported by ELIOT HOWARD as a result of his researches on the warblers, and by BEEBE from his observations on the pheasants. But I do not wish to go into the objections here except to refer to what appears to me to be an outstanding one, which I can myself confirm, namely, that many birds are already paired before they begin their display. Thus, the rook undergoes considerable sexual posturing every breeding season, although the evidence shows that the male and female pair for years together, if not for life (YEATES, 1934). Professor RAVEN (1936), to whom I am greatly indebted for much relevant information, tells me that at the herring-gull colony on Godfrey Island off St. Ives the birds may remain paired for years. His informant was Mr. J. W. LEWIS, one of the lighthouse keepers, who kept careful watch for eight years and reported on the birds, many of which he knew individually. And herring-gulls undoubtedly show some display. The gannet also displays elaborately, as will be recollected by those who have seen HUXLEY's remarkable film (1935); yet KIRKMAN states that the gannet pairs for life. So also grey-lag geese are known to be paired before the breeding season, and other species of geese are said to pair for life (PYCRAFT, 1923). There are, as is well known, a great many

other birds which do this, and HUDSON (1919) has shown that even in gregarious species like the starling, there is evidence that the birds pair for life ; yet most species, if not all, show some sort of display. HINGSTON (1933) states that ducks pair off within the flock during the winter, whereas they may be seen displaying in the spring. In the warblers, buntings, and other passerine birds which form territories in the spring, the male takes up his position and then the female follows him, and it is not until the birds have already associated in pairs that posturing begins and it often takes place during the time of nest building. Sometimes, as Lord TAVISTOCK has found with birds in captivity, coition precedes nest building, and it is probable that in such cases where it takes place so long before the laying of the eggs its significance lies in stimulation rather than in fertilization, for the process is repeated later and often more than once while nest building is proceeding. A further point of interest is that sexual stimulation by display is often mutual, as seen in the simultaneous bobbing up and down of the head by both the drake and the duck, and in the far more elaborate display by both male and female during the courtship of the great-crested grebe, as described by HUXLEY.

The biological advantages of securing an effective synchronization of the male and female reproductive processes, and more particularly in relation to the time of ovulation, become very apparent when one considers the high degree of temporary infertility which animals may show in cases where the correlation concerned is imperfect. It is known that in some mammals ovulation is not always coincident with oestrus. Thus, DEANSLEY (1935) has shown that in the stoat ovulation cycles may precede oestrous cycles, that is to say, that outside of, or early in, the sexual season, the female stoat may ovulate on successive occasions before the oestrous periods commence. The same lack of correlation occurs also in the sheep, as has been shown by GRANT (1933), but in both these species at the later periods of the sexual season ovulation takes place within the phase of oestrus. These are instances of "disharmony" in functional adaptation, of which the reproductive system affords other examples. Moreover, at the end of the sexual season in the sheep, it sometimes happens that oestrus occurs without ovulation, as though the stimulating power of the ewe were insufficient to induce the process in the absence of the ram (MARSHALL, 1922). Nevertheless, the degree of sterility in the sheep from all causes, as estimated by HEAPE (1906), is not more than 6.76%.* In the horse, on the other hand, there is an exceptionally high degree of sterility, the reports of the Royal Commission on Horse Breeding showing that in any one year as many as 40% of mares in this country fail to have foals. Statistical information subsequently obtained points to the percentage of sterility being at least as great in recent years (according to SANDERS (1926), 50%). What is almost certainly the explanation of this unduly high percentage of temporarily or permanently sterile mares has been supplied by HAMMOND (1935), as the outcome of his studies on the oestrous cycle. Unlike the sheep, which has an oestrous period of a day or less, the mare often remains in a

* This percentage figure includes abortion.

condition to receive the stallion for fully a week and sometimes for even longer. Ovulation generally occurs towards the end of the period, and most commonly about 24 hours before the end. Since, assuming that the released gametes of the horse are capable of conjugating for approximately the same duration of time as with the rabbit (in which the times have been ascertained experimentally), that is, for about 30 hours for the spermatozoa and six hours with the ova, it follows that service (which may take place at any time within the limits of oestrus) is often sterile, owing to the gametes losing their power of conjugation before it is possible for them to meet. This most frequently happens to the spermatozoa when the mare is served early in oestrus and many hours or even days before ovulation.

Thus, the importance of an effective synchronization between certain of the sexual processes is illustrated negatively by the condition which we find in an animal under domestication and not subject to the influence of natural selection. And we may conclude that in birds, if not in other animals, the significance of sexual adornment and display lies in their race-survival value, the pair of birds which are most effective in mutual stimulation having an advantage over the others in the perpetuation of the species or variety ; that is to say, we are dealing with a special case of natural selection.*

CONCLUSION

I may now sum up. Speaking generally, there is an internal rhythm of reproduction depending primarily upon the alternation of periods of rest and activity ; in correlation with this rhythm hormones are periodically elaborated by the gonads and act upon the accessory organs and secondary sexual characters. But in the higher animals, the internal rhythm is brought into relation with seasonal changes and other external environmental phenomena, these not merely conditioning the metabolic processes (as they do also in all or most of the lower animals, as well as in plants) but, in part at any rate, acting exteroceptively through the nervous system and probably through the hypothalamus upon the anterior pituitary and thence upon the testis or ovary. In the bird and in the male mammal the hypophyseal and gonadal levels of activity tend to rise and fall together, but in the female the matter is complicated by the occurrence of pregnancy (or pseudo-pregnancy) and in polyoestrous species by the repetition of short dioestrous cycles within the sexual season. In these female cycles there are two main phases, the oestrous or follicular and the luteal, and their repetition in the absence of pregnancy is controlled by the pituitary and ovary acting and reacting upon each other. The condition of pregnancy causes the anterior pituitary to react differently and to prolong the duration of the corpus luteum. Apart from pregnancy, however, both the longer and the shorter cycles are liable to considerable modification by exteroceptive stimuli which play upon the pituitary through the nervous system. The

* Reference may be made here to those species of animals such as the elephant, which lives perfectly healthily in a state of domestication or captivity, and sometimes to a great age without being able to breed. It may well be that this failure in generative function is due to the animals not receiving the appropriate kinds of exteroceptive stimuli.

sequence in the bird's cycle is also frequently interfered with by various kinds of exteroceptive stimuli which control ovulation and the related processes. We may conclude, then, that in all the higher animals sexual periodicity, while conditioned by the environment, is regulated in its successive phases by the combined integrative action of the nervous and endocrine systems.

The primary periodicity is a function of the gonad, the anterior pituitary acting as a regulator, and the internal rhythm is adjusted to the environment by the latter acting on the pituitary, partly or entirely, through the intermediation of the nervous system. The further fact, however, must not be overlooked, namely, that in the absence of the anterior pituitary the functions of the gonad fail, so that the pituitary, in common with the other endocrine organs, conditions the metabolic processes which are essential for reproduction.

POSTSCRIPT

Exactly parallel to the case of the sheep which, on being imported into South Africa, adjust themselves to the southern hemisphere seasonal cycle, is that of the red deer which were introduced into New Zealand. I have not been able to find any published records relating to the importation of the deer, but Lord LATYMER (1936), to whom I wish to express my indebtedness, has kindly given me some important information. Red deer were first sent to Otago about fifty years ago. Lord LATYMER was informed in New Zealand that the first calves were dropped in November, about two years after the mothers had been turned out, but that the change over may begin almost immediately after arrival. Now the rut begins about 20 March (in autumn) and the calves are dropped in the following November or December. Lord LATYMER states that deer have been imported into New Zealand from various sources besides Scotland—from Warnham Court and other English parks (the Rakaia herd being descended from Central European deer)—but that now, wherever they came from, they all begin roaring about the third week in March. As is well known, red deer, like Scottish and other breeds of sheep, in Europe rut in autumn when the daylight is diminishing.*

* I am indebted to Dr. Roux for further information concerning a flock of Southdown sheep transported to a farm near Cradock, Cape Province, South Africa. A breeder imported 21 Southdown ewes which arrived on the farm towards the end of January, 1933. The ewes were in lamb and the first ewe lambed down on 23 January. The others lambed within one month of that date; only one ewe out of 21 was not pregnant. The lambs were duly weaned and the ewes put to the ram on 21 May, 1933. The ewes began to lamb on 15 October; all the ewes lambed. The ewes were put to the ram again on 20 April, 1934, and they lambed in September, 1934, except two, which were late; these lambed on 15 November and 14 December. The ewes were put to the ram again towards the end of March, 1935 and the first lamb was born on 27 August; all lambed within 40 days. The ewes were put to the ram again about the middle of March, 1936; they started to lamb on 13 August, and within 19 days all the 21 ewes had lambed. Thus the ewes lambed regularly during the spring months in South Africa. Southdown sheep in Britain have only one breeding season a year, lambing in spring. It is apparent from the record given above that the ewes lambed regularly also in the spring in South Africa. Thus the conversion of the time of the sexual season was very rapid since it took place immediately after the first lambing in South Africa.

DR. ZUCKERMAN (1936, B) informs me that the spotted deer of India (referred to on the authority of PYCRAFT as having changed its breeding season in captivity in Europe) breeds throughout the whole year and has no season for changing its coat. Dr. ZUCKERMAN states also that with the Thar (*Hemitragus jemlaicus*) in the Zoological Society's Gardens the breeding season as the years went on gradually became later. In the twenty-years period from 1891 to 1910 the peak of births as shown in frequency curves was in the end of May and in the following twenty-years period from 1911 to 1930 it was in the first half of July.*

LACK (1933, B) states that with the Arctic terns on Bear Island the time for ovulation and egg-laying seems to be controlled by the condition of the nesting site. If this is not in a fit state breeding is postponed although the gonads are fully developed, and in a late season the regression of the gonads may set in before the site has become suitable. LACK concludes that with this species, therefore, breeding is controlled partly by the nervous system.

MURPHY (1936) in a book just published on the oceanic birds of S. America, states that in the Atlantic equatorial isles Fernando de Noronha and St. Paul Rocks, the majority of the fowl apparently has no breeding season, for eggs and young may be found in any month of the twelve, but that the sooty tern is an exception. With this species there is evidence that the nesting season on Ascension begins a little earlier in each successive year, and that the change in time is sufficient to make the birds breed on the average four times within three years. It would seem as if in this species breeding periodicity depended entirely upon an internal sexual rhythm unaffected by seasonal exteroceptive factors. MURPHY also records some facts about the periodic breeding of certain species of penguins which are in striking contrast to the great majority of birds. The Peruvian and African penguins, that is, the two members of the genus *Spheniscus* which, coming presumably from the south, have invaded warm-temperate and tropical environments, may have two broods of young, nearly the whole of the year being occupied in breeding operations. KEARTON (1930), says that the African penguins have mating seasons in February and in September. According to LEVICK (1914), the black-footed penguins in the Zoological Gardens can breed several times a year. It would appear that the penguins are almost or quite unique among birds in having freed themselves from external seasonal influences. In this respect they are comparable to the domestic dog among mammals. MURPHY also gives many instances of sea birds of various kinds which seem to have a "continuous breeding season" under uniform conditions.

BAKER (BAKER and BAKER, 1936), in a recent paper on vegetable cycles (with special reference to the New Hebrides), shows that trees and plants are similar to

* Lord TAVISTOCK informs me that the spotted or "Axis deer does *not* adapt itself to our seasons. No tropical deer does. As the Axis breeds and sheds its antlers at *all* times of the year, spring- and summer-born fawns do better in Europe and have more chance of survival, unless protection is afforded, than winter ones. In Europe this deer grows a winter coat and sheds it in spring in the normal way." Lord TAVISTOCK suggests that the later breeding of the Thar as the years went by, was probably a nutritive effect.

animals in that they sometimes have specifically inherent flowering and leaf-bearing cycles which are independent of the environmental periodicity, or they may adjust themselves to the seasons, the latter condition being more usual.

The following additional observations pointing to courtship as a factor in ovulation in birds should also be referred to. HUXLEY (1923) states that the red-throated diver undergoes mutual sexual display which he interprets as being of a stimulating nature. The birds are already paired up in the winter months long before the display, and they possibly pair for life. Lastly, HUDSON (1920) records that with the Argentine cow-bird, which like the cuckoo has the parasitic habit of laying in other birds' nests, the males may be seen wooing the females from the beginning of September until the end of January, and in correlation with this extended period of courtship without nest-building or incubation, the number of eggs produced is phenomenally large (probably from 60 to 100), many of the eggs being dropped on the ground and wasted.

Further observations by the Marquess of TAVISTOCK on the breeding habits and periodicity of certain species of birds in captivity are recorded above in a footnote (p. 439).

As to whether the formation of gonadotropic substances (anti-hormones), such as have been produced experimentally in the serum of animals by COLLIP (1924) and others, plays any part normally in the periodicity of oestrus, is a question upon which there is at present no evidence. (See also ROWLANDS, 1936.)

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XII—Factors Affecting the Amount of Infection Obtained by Aphis Transmission of the Virus Hy. III

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(Communicated by SIR JOHN RUSSELL, F.R.S.—Received 3 April, 1936)

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I—INTRODUCTION

Much of the information which can be obtained about a plant virus agent is ultimately derived from the quantity as well as the type of the infections resulting from inoculations to suitable host plants. The number of infections obtained does not depend solely on the nature of the particular virus concerned. It is dependent on other variable factors, such as the efficiency of the means of infection introducing the virus, the susceptibility of the plants receiving it, and the concentration of the virus in the source from which it was obtained. In this paper an attempt has been made to estimate the effect of some of these variables on infection by insects.

The experiments were carried out with the virus Hy. III (HAMILTON, 1932) which has the following characteristics listed according to the key characters suggested by JOHNSON and HOGGAN (1925) :

Transmission—Mechanically and by the aphid *Myzus persicae* Sulz. into various Solanaceous plants.

Longevity in vitro—Less than 6 days (crude extract).

Thermal Death point—Below 60° C. („ „).

Potato (*Solanum tuberosum*)—Not susceptible.

Cucumber (*Cucumis sativa*)—Not susceptible.

Tobacco (*Nicotiana tabacum*)—Susceptible.

Symptoms in Tobacco—Intense yellow mottling with tendency to strong dark green vein bands. Considerable stunting and occasionally malformation and necrosis.

In its physical properties and transmission by *Myzus persicae*, Hy. III virus appears to be similar to the cucumber mosaic used for aphid experiments by HOGGAN (1933) and by DOOLITTLE and WALKER (1928), and possibly to potato virus Y of K. M. SMITH (1929 and 1933). In its host range it differs slightly from these.

Hy. III was found to be particularly suitable for the type of experiment which it was proposed to undertake. The symptoms are very characteristic and easy to recognize from the earliest stages, which appear 6 days after inoculation in summer and about 8 or 9 days after in winter. The symptom picture does not vary between summer and winter except that in winter there is rather more stunting and necrosis which, when the inoculated plants are seedlings, frequently causes death. The virus has remained standard in all its properties for six years, during which it has been constantly sub-inoculated through different species of plants and has never been renewed from any outside source.

II—METHODS

1—Insectary and Glasshouses

The glasshouses were insect-proofed chambers size 10 feet by 10 feet, heated by hot water pipes. The range of temperature is indicated by the following daily averages for 1933–34 : maximum, April to September, 29–17° C., October to

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March, 15·73° C. ; minimum, April to September, 15·73° C., October to March, 12·94° C.

The insectary was also insect-proofed and was heated by thermostatically controlled electric units, average daily temperature April to September—maximum 21·05° C., minimum 15° C. ; October to March—maximum 25·53° C., minimum 15·28° C. ; average relative humidity April to September—maximum 76·32%, minimum 59·25% ; October to March—maximum 77·27%, minimum 63·32%.

2—Insects and Culturing Methods

Radish and turnip plants were used for stock cultures of *Myzus persicae*, with occasional *Hyoscyamus* to increase the vigour of the colonies, but not for immediate use as *Hyoscyamus* is susceptible to the virus. In November, December, and January, if large numbers of insects were to be used, it was found necessary to lengthen the hours of daylight by 2½ hours. Cultures so treated were kept either under a 500-watt electric lamp in a water-cooled glass-topped cage about 18 inches from the light, or else completely uncovered about 3 feet from it. The artificial light was more effective if the plants were uncovered, but this was only possible when the insectary was not being used for any other purpose.

Culturing on to infected and healthy plants was done with small camel-hair brushes. Single aphids and small numbers were placed directly on to the leaf, but, so far as possible, contact between leaf and brush was avoided. When larger numbers were used the insects were dropped into the top of lamp glass cages with which the healthy seedlings had previously been covered, and on these occasions single aphids were treated in the same way as the larger numbers. The tops of the cages were sealed with cellophane covers.

Aphids were infected with the virus by feeding on single leaves detached from the plant, which were placed upright in damp sand, and covered by open ended glass tubes (3 inches × 1 inch). The aphids were inserted into the tubes which were sealed by cellophane covers.

The aphids were generally starved a few hours before feeding on infected leaves by placing 50–100 at a time into cellophane covered petri-dish bases. They were introduced through a small hole in the cellophane cover which was afterwards sealed, and the whole covered by the petri-dish lid to keep it moist. Thus the time occupied by collecting aphids from the stock plants (2–3 hours when large numbers are required) was eliminated from the actual time of culturing on to the infected leaves, and the insects were all in approximately the same condition and equally ready to feed. Single aphids were removed from the plants at the ends of the feeding periods with clean camel-hair brushes ; larger numbers were sprayed with a mixture of 0·15% pure nicotine in a 1·00% solution of soft soap.

3—Plants

(a) *Difference in Susceptibility between Hyoscyamus and Tobacco*—Although the virus My. III occurs naturally in *Hyoscyamus*, this plant was found unsuitable for experiments

made at different seasons of the year because the seeds could not be persuaded to germinate between October and March. However, as *Hyoscyamus* was used in many of the earlier weekly experiments, it became necessary to find some means of comparing the results obtained with those of experiments in which tobacco plants were used, in order to find out whether any large error had been introduced by a difference in susceptibility of the two species. The method used was to continue for the following spring and summer with random groups of tobacco and *Hyoscyamus* plants in each experiment. The percentage infections obtained for the two species during this period are given in Table I.

TABLE I—DIFFERENCE IN SUSCEPTIBILITY OF HYOSCYAMUS AND TOBACCO

	Plants used	Plants infected	Percentage infection
Tobacco	1102	573	52.00
Hyoscyamus	838	365	43.58

Tobacco was found to be slightly more susceptible than *Hyoscyamus*, the difference between them in percentage infection being $8.44 \pm 2.34\%$. The standard error was determined from the weighted means of the 31 repetitions, as the numbers of each plant were not equal in each experiment.

The remainder of the experiments were carried out with tobacco plants only.

(b) *Comparison between Hyoscyamus and Tobacco as Sources of Infection*—Material for the above experiment was obtained by feeding the aphids for alternate weeks on infected *Hyoscyamus* and tobacco plants, so that it was possible to arrange most of the results according to source of infection as well as for differences in susceptibility. There is no apparent difference in efficiency between the two species as sources of infection (Table II).

TABLE II—DIFFERENCE BETWEEN HYOSCYAMUS AND TOBACCO IN EFFICIENCY AS SOURCES OF INFECTION

	Infection into	Plants used	Plants infected	Percentage infection	Mean
Infection from tobacco . . .	Tob.	500	253	50.6	49.9
	Hyos.	375	177	48.9	
Infection from Hyoscyamus .	Tob.	478	256	53.55	47.82
	Hyos.	361	152	42.10	

(c) *Comparison between Tobacco Leaves of Different Ages as Sources of Infection*—The infected plants on which the aphids were fed were all of the same age and inoculation date for each experiment, and were themselves aphid-infected. In practice, infected plants from one experiment were used as sources of infection for the experiment of

the following fortnight, and when the weekly series was broken arrangements were made, whenever possible, to have extra infected plants at the right stage of development.

In selecting leaves for aphid feeding there were two main considerations. In the first place the aphids prefer smooth, hairless leaves, and it is convenient for them to be small. The second consideration was that plenty of virus should be available to the aphids.

For ease of feeding the older leaves were more suitable, as young infected leaves are often malformed and very hairy. For convenience of handling it was essential to use plants which had not developed beyond the stage of the fifth or, at the most, the sixth true leaf.

The relative virus concentration in the different leaves on which the aphids could be fed was estimated from the results of mechanical inoculation. The plants selected as sources of inoculum had been originally inoculated on the first true leaf at the stage when only three leaves were developed, because this was the standard age for seedlings used in all aphid infections.

Three different groups of infected plants of different ages were used for experiments I, II, and III (Table III). Inoculations were made on to healthy tobacco plants using extracts of leaves taken in order of age from these groups of infected plants. Thus the first true leaf provided extract No. 1, the second extract No. 2, and so on to the youngest leaf, the number of extracts depending on the number of leaves which had been developed since the original inoculation.

Counts were made of local starch lesions by HOLMES's method (HOLMES, 1931), and in this way much smaller numbers of plants were needed than would have been required to obtain the same information by means of aphid infections.

The juice was extracted without addition of water to the leaf pulp and the dilutions were made up from these neat extracts.

The inoculations were made by rubbing with the finger-tip on to half-leaves, about 0.1 c.c. being used for each inoculation. The other half-leaf was rubbed with the same quantity of a control juice composed of equal quantities of each of the extracts used in the experiments. These control halves were used in an attempt to eliminate some of the differences in susceptibility between leaves and plants, because YODEN and BEALE (1934) found that there is a high correlation between half-leaves. As will be seen below, no advantage was derived from this.

The method of counting and preparing for lesion counts was slightly altered from that of Holmes, in accordance with the special features of the virus used. Hy. III in tobacco gives clearly defined starch lesions varying in appearance from a minute solid spot in the young lesions, through a stage in which 1, 2, or 3 concentric rings are present, which later become rather blurred and diffuse. The best stage for counting is that of the first concentric ring, which occurs on the fourth day after inoculation in summer, and in winter on the fifth or even the sixth day. At 5 p.m. on the fourth day from the date of inoculation the inoculated plants were placed in a cool dark room to remove starch from the leaves, and at 9.0 a.m. on the following day the

inoculated leaves were detached, killed by immersion in boiling water, and decolorized in alcohol. They were then cleared in distilled water for 24 hours and placed for 10 minutes in an aqueous solution of iodine and potassium iodide. After washing, the leaves were floated in water on glass trays and the lesions counted by transmitted light.

Table III shows the results given as lesion counts per half-leaf for each treatment. The last column gives the experimental figures with the number of lesions on the control halves adjusted to a constant value by means of the analysis of covariance (*see* FISHER, 1935, p. 167).

TABLE III—RESULTS OF INOCULATIONS FROM LEAVES OF DIFFERENT AGES

Experiment No.	Number of leaf used as source of inoculum	Mean number of lesions per half-leaf.		
		Control	Experimental	
			Actual	Adjusted
I (25.7.35)	1	47.45	5.18	4.19
	2	32.27	2.36	2.55
	3	27.90	4.81	5.34
	4	31.09	3.63	3.91
	S.E. mean of 11 observations		± 0.952	± 0.754
II (30.10.35)	1	12.00	6.67	7.07
	2	9.00	0.33	1.60
	3	16.33	13.67	12.82
	4	11.33	35.00	35.60
	5	18.33	17.00	15.57
	S.E. mean of 3 observations		± 3.10	± 3.111
III (9.8.35)	1	63.56	4.00	3.25
	2	65.56	0.56	— 0.30
	3	30.66	4.56	5.64
	4	49.16	9.00	9.06
	5	50.00	4.16	4.17
	6	41.83	2.00	2.52
	S.E. mean of 6 observations		± 1.514	± 1.404
			Control	Experimental
Dilution for experiments I and III			1/100	1/1000
Dilution for experiment II			1/100	1/100

The results for experiments II and III show a concentration of virus for the fourth leaf which is significantly greater than those for the other leaves used. The difference between leaves for experiment I is not quite significant, but the adjusted figures agree with the results of the other two experiments, except that in the younger plants the third leaf gives the highest concentration. In no experiment was the error greatly decreased by the use of the regression on the controls, which

indicates that the increased accuracy obtained was not sufficiently great to justify the use of half the experimental material for control purposes. This point will be discussed in the next section.

These results indicate that the first two leaves of a young plant, though the most satisfactory for aphid feeding, are not the most efficient sources of infection. The third leaf was therefore chosen on all possible occasions, but where the supply of third leaves was not sufficient, a random selection of first and third leaves was used, as the fourth was generally deformed and very hairy, and the second appeared to have a low virus concentration.

(d) *Differences in Susceptibility between Tobacco Leaves of Different Ages*—Experiment II above was designed so that it might also yield some information about susceptibility of the various leaves to inoculation. Such information applies only to mechanical inoculation, and susceptibility to aphid inoculation will not necessarily be the same in all respects, but some factors at least are probably common to both methods of infection.

A preliminary grouping to find the principal source of variation *within plants* was made by dividing the leaves into blocks according to age. This arrangement gave the following total numbers of lesions for all treatments :—

3rd leaf		4th leaf		Total	
Experimental	Control	Experimental	Control	Experimental	Control
98	1083	49	741	147	1824

The analysis of variance for the experimental figures :—

	D.F.	Mean Square
Blocks (3rd leaf v. 4th leaf)	1	66.69
Blocks \times Treatments	5	8.58
Treatments	5	48.65
Error	24	13.75

shows that the mean square variance for blocks is significant, and this is true also for the controls and for the adjusted figures.

In order to obtain more exact information on this point and also, though this was not of immediate interest from the point of view of aphid infection, to examine the correlation between half-leaves, a uniformity trial was made using the same virus extract for all treatments. In this experiment 10 similar plants were selected and independent halves of the second, third, and fourth leaves rubbed at random with a dilution of 1/100 infected plant extract. The randomization was done by cutting from a pack of 60 numbered cards, one for each of the 60 half-leaves. The results are given in Table IV.

The upper part of Table IV gives the results for each inoculation on the third and fourth leaves. No figures were obtained for the second leaves as they took a very severe infection which resulted in large areas of necrosis so that accurate counts were impossible. The lower part of the table gives the analysis of variance on a

plant basis for the totals of 4 figures. Comparison of the mean squares resulting from this analysis brings out the following points :

1. The third leaf has given a significantly higher mean number of lesions than the fourth, the difference being 67.4 with a standard error of ± 22.47 ($= \sqrt{504.75}$). In view of this it appears that advantage would be gained in an experiment by

TABLE IV—RESULTS FOR LESION COUNTS ON THIRD AND FOURTH LEAVES

Plant number	Third leaf		Fourth leaf		Difference (Third leaf v. Fourth leaf)
	Right half	Left half	Right half	Left half	
1	56	73	32	50	47
2	33	32	16	35	14
3	47	58	22	48	35
4	58	48	47	50	9
5	72	68	16	21	103
6	75	56	66	83	-18
7	40	60	29	49	22
8	76	101	17	39	121
9	179	106	23	18	244
10	77	63	18	25	97
Total	713	665	286	418	674

Analysis of variance (on a plant basis—total of 4 figures).

	Degrees of freedom	Sum of squares	Mean square
<i>Between plants</i>	9	31,805.6	3,534.0
<i>Within plants between leaves.</i>			
Third leaf v. fourth leaf	1	53,286.4	53,286.4
Remainder	9	45,427.6	5,047.5
<i>Within leaves between half-leaves.</i>			
Right half v. left half	1	705.6	705.6
Remainder	19	19,454.4	1,023.9
Total	39	150,679.6	4,023.6

keeping these leaves in separate blocks so that the average differences between them might be eliminated from the treatment comparisons.

2. This is supported by the fact (obtained from comparison of the mean square variance *between plants* with the remainder) that the variation *between plants* is no greater than *within plants between leaves*, so that nothing would be gained by restricting blocks to single plants.

3. There is apparently no difference between right and left halves of leaves as is shown by a comparison of the two mean squares for *within leaves between half-leaves*.

The variation *between leaves* is, however, significantly greater than that *within leaves* so that there is a correlation between the numbers of lesions on halves of the same leaf; but it has already been shown that this correlation is not sufficiently great to increase the accuracy of experiments where corresponding half-leaves are used as controls. Much greater advantage would probably have been gained by using each half-leaf independently for experimental inoculations, thus doubling the number of observations. Similarly, SAMUEL, BEST, and BALD (1929) found, for spotted wilt disease of tomatoes, that no advantage was gained by the use of control half-leaves in this type of experiment. This may be due to the fact that with rubbing experiments, it is inexpedient to follow each experimental inoculation with its control, because of the danger of accidental infection. Consequently the experimental and control inoculations were done in separate groups, which tends to decrease the variation between leaves within plants, and between plants, at the expense of that between half-leaves.

As the results showed a considerable difference in susceptibility between leaves of different ages, it was necessary to use the same leaf consistently for aphid infections whenever possible, or else to use efficient randomization between different leaves. The older leaves appeared to be the most susceptible and as they were also the most suitable morphologically, aphids were fed on the first true leaf or a random selection of first and second. This does not apply to occasions on which large numbers of aphids were fed, or to those in which the aphids were dropped into the glass cages. In these experiments the aphids selected their own feeding places.

III—APHID INFECTIONS

1—Seasonal Variation

A possible source of error when experiments are done at different times of the year is seasonal variation, and in glasshouse conditions there appears to be a considerable seasonal effect on the percentage infection produced by *Myzus persicae* with the virus Hy. III.

Fig. 1 shows the weekly percentage infection obtained out of 30 plants per week for 14 months, 1933–34. The results for 5 and 10 aphids were used together. The total hours of sunshine for the same weeks are also shown.

A winter maximum of percentage infection is reached which continues from the end of October to the middle of January and corresponds to the period of least sunlight. The positions of the points suggest a negative correlation between deviations from the smooth general trend of the two curves, indicating that the hours of sunshine for the week in which the infection took place, or possibly in the previous week, have a direct effect upon the number of infections obtained. This, however, was not significant. There appeared to be a negative correlation with average mean daily temperature for the current week, though this was not quite significant. (Variance due to regression 395.4; residual variance 189.84.) This effect

was probably due to the lengthening of the penetration time (*see* p. 475) by a fall in relative percentage humidity, resulting from high temperature, and in any case only accounts for a very small fraction of the variance.

Fig. 2 gives the two curves representing the mean weekly percentage infection for 5 aphids per plant over the period October to March for the years 1933-34, and 1934-35. The gradual rise to a maximum for December and January and a drop towards the following spring is clearly shown in both curves, so that the effect appears to be a general one and not peculiar to 1933-34. The 1934-35 curve, particularly the autumn part of it, is smoother than that of the previous year. No corresponding

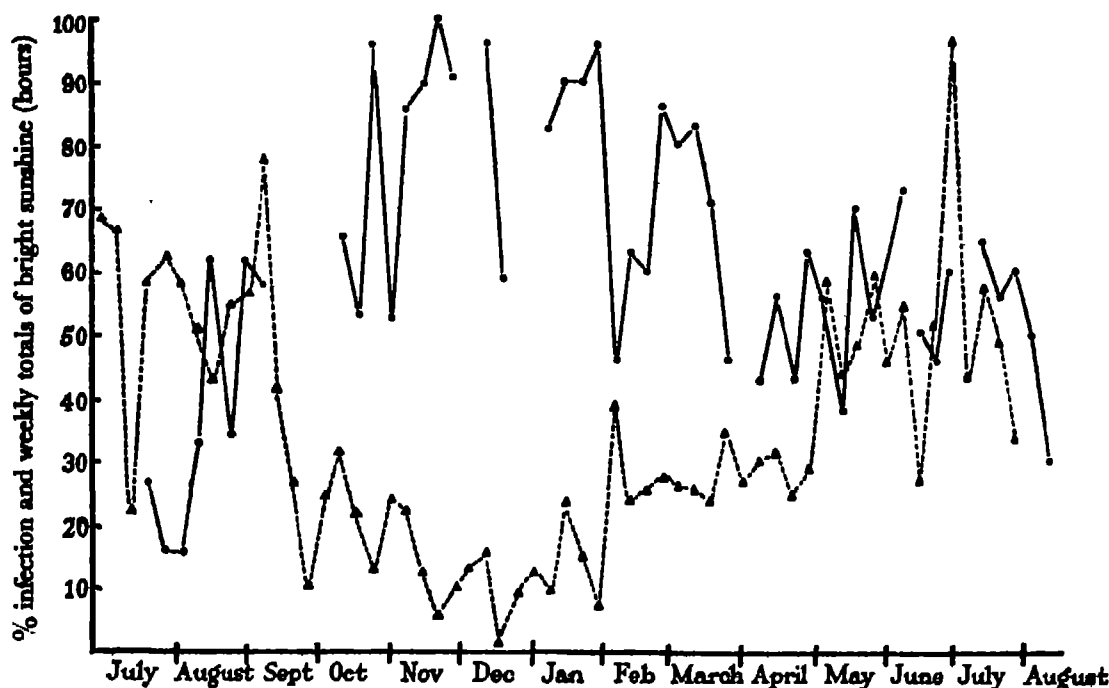


FIG. 1—Seasonal variation in percentage infection, with weekly sunshine curve. • % infection ; ▲ weekly sunshine curve.

differences were recorded for meteorological conditions, and this result is probably attributable to improved technique and greater experience in the measures necessary for controlling conditions in the insectary.

The two curves do not correspond particularly well in their weekly variations except at one point, namely, the large fall in percentage infection between the second and third week in December. This follows closely on periods, terminating on 27 November, 1933 and 4 December, 1934, when degeneration of the insect cultures from lack of light, or indirectly from starvation due to etiolation of the food plants, necessitated a break in the experimental series and artificial light had to be used to restore them. This upset the experimental routine so that at first rather old and then very young plants had to be used as sources of infection, which possibly accounted

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for the observed fall. The use of artificial light on the aphid cultures did not otherwise appear to affect the general trend of percentage infection.

These results show a clear seasonal variation in percentage infection, and this variation occurs for all numbers in aphids used per plant. The following figures give the annual range in percentage infection for each aphid number. They are taken from the monthly averages as the weekly totals show a very wide variation. The range is greater for the smaller aphid numbers than for the larger ; this type of

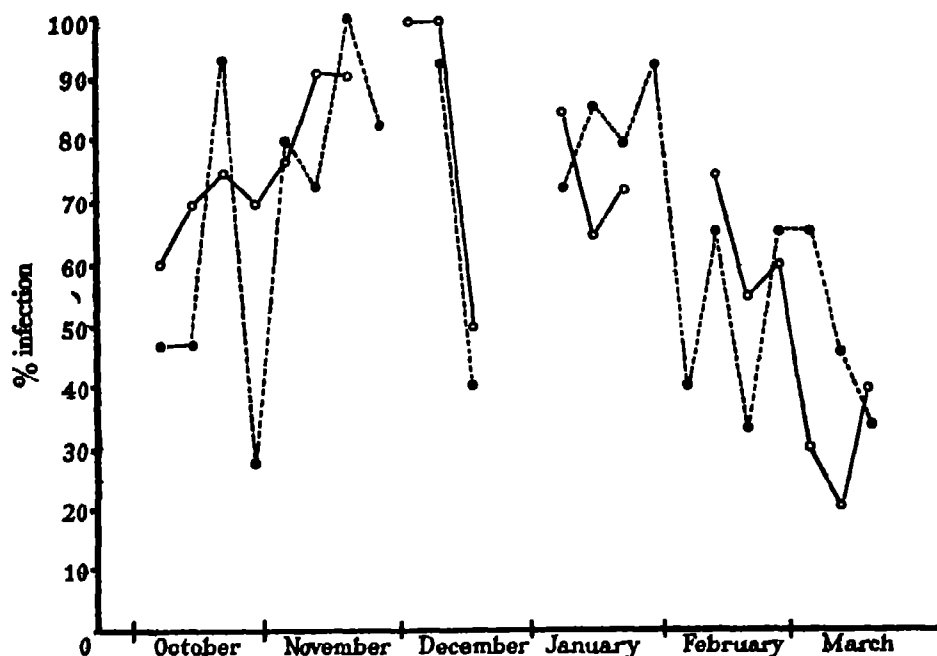


FIG. 2—Winter and Spring variation in percentage infection for two consecutive years, 5 aphids per plant. • 1933-34 ; ○ 1934-35.

variation is also found in other experiments when different aphid numbers are used (see page 471).

For 1 aphid	between 5 and	40%.
„ 5 aphids	„ 20	„ 80%.
„ 10 „	„ 40	„ 95%.
„ 20 „	„ 75	„ 100%.

The absence of significant correlation between individual weekly totals and meteorological conditions is probably due to the interaction of many other factors which tend to mask or nullify these effects ; for example :—

1. Biological errors due to variation in the condition of the aphid cultures and in technique.
2. The inadequacy of hours of sunshine as a criterion of light effect. Total radiation in the insectary and glasshouses would probably have been better, but this was not recorded.

3. The possibility that susceptibility to infection is increased as rate of plant growth increases. Increase in hours of daylight from the beginning of March should, according to the general trend of the curve, cause a decrease in percentage infection but coincides with the onset of rapid spring growth in the plants, and the general descent of the curve would be checked until the flush of spring growth ceases at the end of June. This would account for the two periods of minimum percentage infection shown by the curves in March-April, and later in July-August.

2—Effect of Number of Aphids Used per Plant

HOGGAN (1933), using cucumber mosaic, has shown that the amount of infection obtained with *Myzus persicae* as a vector varies with the number of aphids used. With Hy. III the results from 240 infections in tobacco for 1, 5, 10, and 20 aphids are given in the top row of the upper part of Table V, and it can be seen that the same is true for this virus.

TABLE V—DISTRIBUTION OF INFECTION ON 960 PLANTS

No. of aphids per plant	1	5	10	20
No. of plants (out of 240) infected	28	127	163	190
Expected number, susceptibility constant	24	99	157	211
Expected number, susceptibility varying	25	100	150	199

The figures were obtained from the results of 16 weekly experiments carried out between April and September, 1934. The 60 plants used in each experiment were divided into batches of 15 which were treated alike in respect of aphid number but were also subjected to different feeding times in batches of 3 plants.

If the probability of infection, p , by a single aphid were constant and independent of whether other aphids were feeding on the plant, so that the probability of non-infection, q , by this aphid is $q = 1 - p$, then the probability of non-infection by x aphids all feeding simultaneously would be q^x , and consequently the probability of infection would be $1 - q^x$, since only when none of the x aphids produces infection will the plant as a whole escape infection.

The second line of Table V shows the expected number of infected plants with $p = 0.1005 \pm 0.0047$, which is the value given by the maximum likelihood solution. The agreement is not perfect, there being too few infected plants in the 20 class and too many in the others, particularly the 5 class.

If the probability of infection varies from batch to batch, similar figures may be deduced, estimating a different value of p for each batch of 60 plants. The last line of Table V shows the expected values obtained on this hypothesis. The observed and expected numbers of infected plants and the corresponding values of p for each batch are shown in Table VI. p ranges from 0.05 to 0.15. The total observed and expected values now agree somewhat better, but there is still evidence of a tendency for the number of infected plants to be lower than expectation for 20 aphids and above expectation for 1, 5, and 10 aphids.

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TABLE VI—DISTRIBUTION OF INFECTION FOR WEEKLY RESULTS OF 16 EXPERIMENTS, FIRST SERIES

Experi- ment No.	1 Aphid		5 Aphids		10 Aphids		20 Aphids		<i>q</i>
	A.V.*	M.L.V.	A.V.	M.L.V.	A.V.	M.L.V.	A.V.	M.L.V.	
1	0	1.0	7	4.6	7	7.7	11	11.5	0.93
2	0	1.5	7	6.1	12	9.8	12	13.2	0.90
3	0	1.2	6	5.1	7	8.5	11	12.2	0.92
4	2	1.8	9	7.1	10	10.8	13	13.8	0.88
5	2	1.8	10	7.1	7	10.8	15	13.8	0.88
6	2	1.2	12	5.1	12	8.5	5	12.2	0.92
7	3	1.5	8	6.1	13	9.8	10	13.2	0.90
8	1	2.2	10	8.3	12	12.0	14	14.4	0.85
9	2	1.4	5	5.6	9	9.2	13	12.7	0.95
10	3	1.0	7	4.6	6	7.7	11	11.5	0.93
11	3	1.5	6	6.1	12	9.8	11	13.2	0.90
12	3	2.1	10	8.0	12	11.7	13	14.3	0.86
13	4	1.6	7	6.6	11	10.3	12	13.5	0.89
14	0	1.6	6	6.6	11	10.3	14	13.5	0.89
15	2	1.8	6	7.1	15	10.8	12	13.8	0.88
16	1	1.5	11	6.1	7	9.8	13	13.2	0.90
Total	28	24.7	127	100.3	163	149.8	190	198.6	

* A.V. = Actual Value ; M.L.V. = maximum likelihood value ; *q* = probability of non-infection by single aphid.

Discrepancies of this nature would arise if the probability of infection varied from plant to plant, as is easily seen if we consider the limiting case in which some plants become infected on exposure to any infection and others are totally immune. If all aphids carried infection, the numbers of plants infected would be equal for all the numbers of aphids per plant.

Such discrepancies might also arise if different aphids carried different amounts of the infective material, as might happen if different leaves of the host plant were infected differently. In the absence of proper randomization, the whole of a batch of 20 aphids might then tend to be highly infected, or only slightly infected, and this would give rise to the type of discrepancy observed. Therefore, a second series of experiments was carried out in the spring of 1935, using larger numbers of plants over a period of 10 weeks so that the material used was more homogeneous, and the experiments less widely separated in time, than in the previous ones.

The procedure of these experiments was as follows :

On each of forty plants one aphid was placed and immediately 10 plants were withdrawn at random, forming the group receiving one aphid only. The remaining 30 plants received four more aphids given singly to each plant in its turn. Ten were withdrawn at random and constituted the 5-aphid group. The 10- and 20-aphid groups were formed in the same way. Thus, over the whole series, 100 plants were

used for each aphid number. The results of the experiments with the expected values calculated from the maximum likelihood value of q for the total figures ($q = 0.828$) are given in Table VII.

TABLE VII—DISTRIBUTION OF INFECTION FOR WEEKLY RESULTS OF 10 EXPERIMENTS, SECOND SERIES

Experiment No.	1 Aphid	5 Aphids	10 Aphids	20 Aphids
1	4	9	10	10
2	2	6	10	9
3	2	2	9	9
4	0	5	7	10
5	3	7	8	9
6	2	9	10	10
7	2	6	10	10
8	1	4	10	9
9	2	6	8	9
10	0	6	9	9
Number of plants infected out of 100	18	60	81	94
Expected number, sus- ceptibility constant . .	17.2	61.1	84.4	97.7

The expected values now fit very closely to the figures obtained so that part of the discrepancy found in the previous series of results has been eliminated by the later modifications in technique and materials.

If the infections were not local and independent but could be caused by the cumulative effect of individually inadequate doses, discrepancies of the opposite type would occur, the spread of the observed values being wider than that of the expected values. An effect of this kind may conceivably be masked by either or both of the causes of disturbance discussed above, but, in the absence of other evidence, each aphid infection may reasonably be assumed to be essentially local and independent.

3—*Effect of Varying Feeding Times on the Healthy Plants*

In these experiments aphids were fed for 12 hours on infected leaves, then placed in batches of varying number on to groups of healthy seedlings, and allowed to feed for varying periods. It was desired to use as large a number of plants as possible for each treatment and this necessitated numerous repetitions at weekly intervals. In order to equalize so far as possible the effect of season and other special conditions over the various repetitions, they were continued, with a few breaks, throughout the year 1933–34. The weekly mean percentage infection gave the data on seasonal variation in percentage infection which was discussed in § III.

The first experiments were carried out with groups of 5 and 10 aphids, between July and September, 1933. For each aphid number 15 plants were used and sets of 3 from each group were sprayed after periods of 3, 6, 12, 24, and 48 hours' feeding.

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The 20-aphid groups were carried on in the same way as the previous ones from September until the end of November, when they were discontinued owing to shortage of aphids. The winter percentage infection curve reached its highest values at this time, and the 20-aphid groups were giving a regular weekly result of 100% infection for all times so that the interruption probably caused little loss of information. The 20-aphid treatment was restarted in February, 1934, when the percentage infection was falling again.

Experiments with single aphids had not been started in July with the 5- and 10-aphid groups because previous experiments done in spring and summer had shown

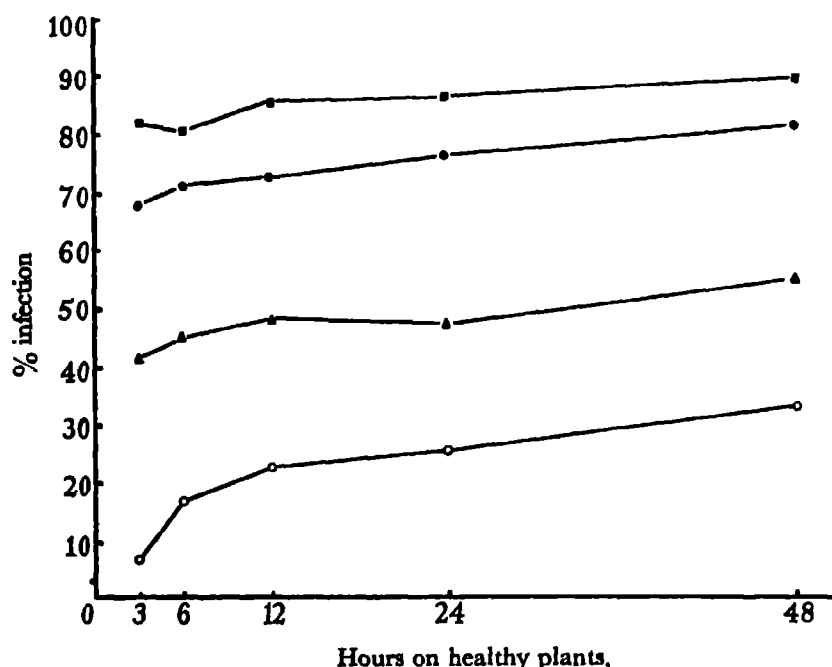


FIG. 3—Effect of increasing feeding periods on the healthy plant for different numbers of aphids.
■ 20 aphids ; • 10 aphids ; ▲ 5 aphids ; ○ 1 aphid.

very small or negative results. When the higher winter percentage infection became apparent in December, 1933, single aphid groups were started and continued until the end of the experiment. They gave relatively high percentage infections in the winter and early spring, the figures for the following summer being small but consistent with the other results.

The results of these experiments are given in Table VIII, and fig. 3 shows the percentage infection plotted against time on healthy plant for each aphid number.

The linear regressions, which measure the average increase in percentage infection per hour, are also given in Table VIII, and they are highly significant.

The increase in percentage infection per hour tends to lessen as the numbers of aphids increase. This is not surprising since for the higher aphid groups many of

the winter results show 100% or nearly 100% infection for all times on the healthy plant.

There does not appear to be any distinct interval of time before infection is possible which could be called an "incubation period", such as is described by KUNKEL

TABLE VIII—EFFECT ON PERCENTAGE INFECTION OF TIMES OF FEEDING ON HEALTHY PLANTS

Hours on Healthy Plant	1 Aphid		5 Aphids		10 Aphids		20 Aphids	
	Plants used	% infection	Plants used	% infection	Plants used	% infection	Plants used	% infection
3	114	6.6	144	41.6	141	68.1	72	81.8
6	114	16.5	144	45.1	138	71.1	72	79.7
12	114	22.6	141	48.2	135	72.7	72	85.5
24	114	25.5	138	47.1	135	76.5	69	86.3
48	114	27.8	138	55.7	135	82.5	69	89.3

Increase in per-
centage infec-
tion per hour*

0.37 ± 0.161

0.27 ± 0.0519

0.30 ± 0.0294

0.18 ± 0.056

* Calculated from the linear regression coefficient.

(1926), STOREY (1928), and others. Their viruses, however, appear to have a different type of relation to their vectors (*see* p. 484). The suggestion of an incubation period in the insect for Hy. III made in my previous paper HAMILTON (1932) was due to small numbers of plants having been used at a season of low percentage infection, so that only their periods of maximum infection at 24 and 48 hours gave positive results.

HOGGAN (1933), using cucumber mosaic, did not find any difference in percentage infection for feeding periods of from $\frac{1}{4}$ hour to 24 hours on the healthy plant. This may be due to differences in the nature of cucumber mosaic and Hy. III, but it is also possibly accounted for by the fact that large numbers of aphids, 10 or 20 per plant, were used so that the increase per hour would be relatively small.

The shape of the curves is of interest in that the upper pair of lines are straight and probably do not pass through zero; the regions corresponding to the shortest times in the lower pair are curved and might conceivably do so.

In order to find out the effect of shorter feeding periods another series of experiments was carried out between September, 1934, and January, 1935. The treatments consisted in culturing groups of 1 and 5 aphids on to batches of 5 plants each and allowing them to feed for periods of $\frac{1}{4}$, $\frac{1}{2}$ (for single aphids only), 1, 3, 6, and 12 hours. Some short term infections had already been done for 5 aphids in the summer of 1934 and these are included in the 5-aphid results. The results are given in Table IX and rise in percentage infection is plotted against time in fig. 4.

For the whole period of 24 hours the slopes are somewhat steeper than was found for the 48-hour periods, the rise for the 1-aphid line being about 1% per hour and

for the 5-aphid line being 0·8% per hour against 0·37% per hour for the 1-aphid 48-hour curve, and 0·27% for the 5-aphid curve. This indicates that the rise is faster for the shorter feeding periods, and that the suggestion of departure from linearity in the region corresponding to these times is probably justified. There is no suggestion from the curves that infection is not instantaneous, for if there were

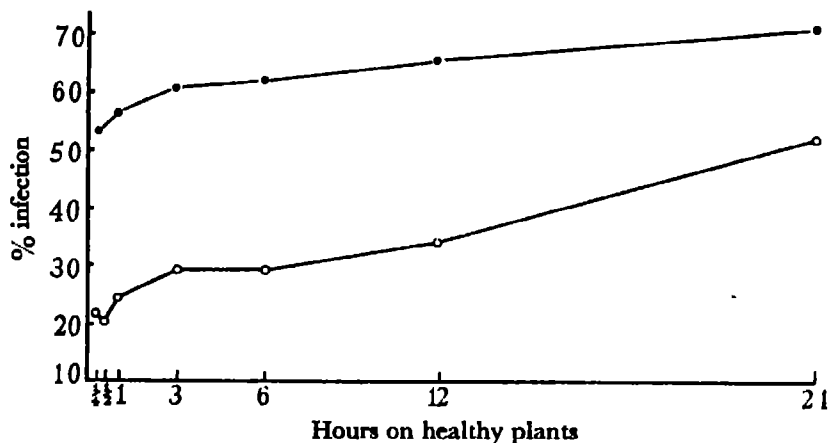


FIG. 4—Effect of shorter feeding periods on the healthy plant. • 5 aphids ; ○ 1 aphid.

TABLE IX—EFFECT ON PERCENTAGE INFECTION OF SHORT PERIOD FEEDING ON HEALTHY PLANTS

Time on Healthy plant	1 Aphid		5 Aphids	
	Plants used	% infection	Plants used	% infection
¼-hour	72	22·2	114	53·5
½-hour	72	20·2	—	—
1 hour	72	26·4	114	56·1
3 hours	72	27·8	111	60·5
6 hours	69	27·5	111	61·3
12 hours	63	34·5	104	66·0
24 hours	69	47·6	104	74·0
Increase in percent- age infection per hour*	1·01 ± 0·107		0·82 ± 0·142	

* Calculated from the linear regression coefficient.

any appreciable lag, zero percentage infection would be shown for a positive value of the time.

The actual percentage figures are higher in fig. 4 than in fig. 3, because most of the infections were done in the winter period, whereas the previous results were totals for a whole year, but the general trend for all curves is the same. It is remarkable

that the increase shows no signs of falling off at the 24- and 48-hour periods ; this may be due to insufficient data or to increased susceptibility of the plants due to aphid injury (see p. 486).

4—Preliminary Experiments on the Effect of Feeding Time on Infected Plants, Short Term Feeding and Consecutive Infection

A further series of infections with single aphids was carried out between January and early May, 1935, to find out the effect of very short feeding periods on healthy plants, and of variation in feeding time on infected plants. Weekly batches of 10 plants for each treatment were used. (The arrangement is shown in Table X.) Each experiment was divided into two halves, half the aphids being fed on the infected leaves for 12 hours (9.30 p.m. to 9.30 a.m.) and half for 3 or 5 minutes. The aphids were immediately cultured on to batches of 10 healthy seedlings which were called the "A" plants. After their allotted periods of 3 or 5 minutes they were transferred directly to a second series of 10 seedlings, called the "B" plants, in order to find out whether they were capable of infecting more than one plant without further access to sources of infection. Consecutive infections are those in which an aphid has infected a second healthy plant after feeding on the first. The aphids were allowed to feed on the "B" plants for 2 hours.

To obtain the 3- and 5-minute feeding periods on both healthy and infected plants, individual aphids were watched through a lens until they had settled into the feeding position ; that is to say, with the abdomen horizontal and close to the leaf, the antennae laid back in line with the body, and the rostrum at right angles to the leaf and touching it. They were only considered as fed when they had held this position continuously for the requisite time.

The time occupied by the aphid in finding a suitable feeding place and settling down to feed, is referred to as the "Penetration Time". For 95% of the aphids, the penetration time was less than 10 minutes, 3% took between 10 and 15 minutes, and about 2% longer than 15 minutes ; but some of these may have been damaged and would not have fed at all. The average penetration time for 560 aphids was 4.88 minutes.

It was noted that penetration time was longer on some days than on others and this variation was found to be negatively correlated with relative humidity in the insectary at the time of feeding. Fig. 5 shows the relation between penetration time and relative humidity at time of feeding. The time chosen for ease of reading the hydrograph record was 10 a.m., but the actual series of cultures took about 1½ hours to complete. The relative percentage humidity was recorded by a recording hair hygrometer.

The linear regression coefficient of penetration time on relative humidity, — 0.129 ± 0.031 , was highly significant and accounted for 52.5% of the variance.

The preliminary results for short feeding periods are given in Table X in which the number of infections obtained from feeding on the first plant of each pair are given in the columns headed A. The columns headed B give the total number of infections

in the second series of plants, and the C columns show the number of consecutive infections in which both A and B were infected. The fact that B infections occur when A is not infected may have been due to failure on the part of the observer to ensure proper feeding on A, but later experiments indicate that this is unlikely, so that a large proportion of these also represent consecutive infections (p. 483).

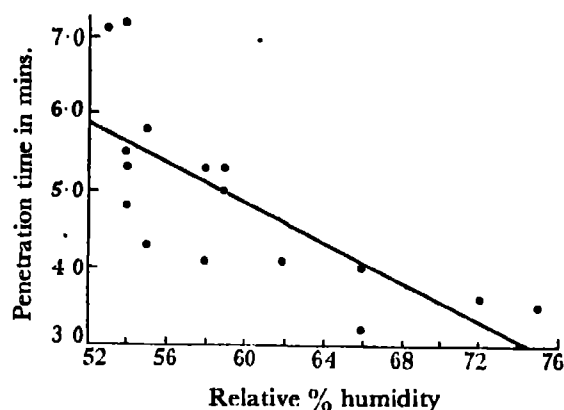


FIG. 5—Effect of relative humidity on "penetration time".

TABLE X—PERCENTAGE OF INFECTIONS AND CONSECUTIVE INFECTIONS OBTAINED BY SINGLE APHID FEEDING FOR VARIED TIMES ON INFECTED AND HEALTHY PLANTS

Feeding time on infected plant	Feeding time on healthy plant A					
	3 minutes			5 minutes		
	A	B	C	A	B	C
3 minutes	74	40	30			
5 minutes				64	40	31
12 hours	18	15	10	28	15	13

Feeding time on the B plant was 2 hours throughout

This preliminary experiment gave a somewhat unexpected result, in that the percentage infection decreased with increasing time on the infected plant, and a very much higher number of infections was obtained for 3 and 5 minutes' feeding than for 12 hours. There is agreement with previous results in the increase in percentage infection with increasing time on the healthy plants.

The aphids show themselves capable of infecting more than one plant consecutively without intermediate access to any source of infection. This is not remarkable in itself, for many insect vectors of virus diseases retain their infectivity for long periods, but it was not in agreement with the results of previous experiments in which two hours' feeding period was allowed on the first healthy plant and in which no consecutive infections were obtained.

Such anomalies might be caused by variation in the probability of infection due to other factors, such as have been described in previous sections of this paper, and it was thought that some advantage would be gained by more comprehensive investigations.

5—*Factorial Experiment*

A second series of experiments was arranged on a factorial design (FISHER, 1935). In materials and technique they were similar to the experiments described in § III, 4, except that 3 plants instead of 10 were used for each treatment and consecutive treatment. Six variants of feeding time on infected and healthy plants were tested giving 36 combinations of treatments, and the whole was repeated for consecutive infections, giving 72 combinations. Thus the total number of plants used in each experiment was 216, and the experiment was repeated for 10 weeks, giving 2160 plants in all. The general arrangement is shown in Table XI in which columns A, B, and C are the same as in Table X.

The six variants of each factor, that is to say the feeding time on infected plants and on the first healthy plants, or "A" plants, were 2 minutes, 5 minutes, $\frac{1}{4}$, 1, 6, and 12 hours. For all the two-minute and five-minute feedings the aphids were watched and the feeding period timed from the moment of penetration. For the quarter-hour feedings the average "penetration time" was allowed in addition to the treatment feeding time, but for the longer periods the penetration time was neglected. On the second series of healthy seedlings, namely, the "B" plants, all the aphids were allowed to feed for twenty-four hours.

Such a long series of cultures and sub-cultures could not be carried out even approximately at the same times of day. The programme of work was, however, planned so as to equalize, so far as possible over the different treatments, the times of day at which these treatments were started. This was most difficult for some of the six- and twelve-hour feedings, which could only be varied by starting some in the morning and feeding till night and others at night and allowing them to feed till morning.

The actual conditions in which the aphids were fed were not very variable, for the humidity within the lamp glass cages varied very little throughout the day, and all the watched feedings were done under small bell jars in which the air was kept at a high relative humidity by means of an atomizer. After dark the feedings were carried on by artificial light. No marked variation in penetration times or in the general behaviour of the aphids was observed for any particular time of the day. The main danger was the possibility of variation in susceptibility of the plants during the course of the day, but any effect on the treatment comparisons was presumably eliminated by altering the times at which treatments were started from one extreme to the other.

The results for the 10 experiments are given in Table XI which shows the number of infections for each treatment, and the totals for all variants of each factor.

The results confirm those of the preliminary experiment concerning short term feeding on the infected plant, and support those already given (p. 473) for increase in

TABLE XI—RESULTS OF FACTORIAL EXPERIMENT

Time on infected plant.	Time on first healthy plant																	
	2 min.			5 min.			15 min.			1 hour			6 hours			12 hours		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
2 mins.	17	11	11	20	8	8	13	4	2	18	0	0	16	1	1	23	0	0
5 mins.	11	7	2	13	10	8	10	0	0	14	0	0	18	0	0	15	0	0
15 mins.	10	4	0	6	3	3	10	2	2	9	0	0	9	0	0	18	0	0
1 hour	3	3	2	3	2	1	2	0	0	2	0	0	7	0	0	4	0	0
6 hours	3	3	1	1	0	0	7	2	0	6	2	2	6	0	0	12	0	0
12 hours	6	4	3	9	4	1	8	3	0	2	0	0	4	0	0	9	0	0
Total	50	32	19	52	27	21	50	11	4	51	2	2	60	1	1	81	0	0
																344	73	47

A = Total number of infections on first healthy plant.

B = Total number of infections on second healthy plant.

C = Total occasions on which A and B were both infected by the same aphid.

infection with time allowed on the healthy plant. Those for consecutive infections show that there is a decrease in consecutive infection with increasing time on the first healthy plant, and this is also in agreement with expectation.

The data from which figs. 6 and 7 are plotted are given as percentages with their means and standard errors in Table XII.

TABLE XII—SUBSIDIARY TABLE SHOWING PERCENTAGE INFECTIONS FOR TIMES ON INFECTED AND HEALTHY PLANTS

		Time on healthy plant						Mean	
		2 m.	5 m.	15 m.	1 hr.	6 hr.	12 hr.		
Time on infected plant	Part I	2 m.	56.66	66.66	43.33	60.00	53.33	76.66	Standard error of Mean ± 3.57
		5 m.	36.66	43.33	33.33	46.66	60.00	50.00	
		15 m.	33.33	20.00	33.00	30.00	30.00	60.00	
	Mean		42.22	43.33	36.66	45.55	47.77	62.22	S.E. of Mean ± 5.03
	Part II	1 hr.	10.00	10.00	6.66	6.66	23.33	10.33	
		6 hr.	10.00	3.33	23.33	20.00	20.00	40.00	
		12 hr.	20.00	30.00	26.66	6.66	10.33	30.00	
	Mean		13.33	16.66	18.88	11.11	18.88	26.66	S.E. of Mean ± 3.50
	Mean of Parts I and II		27.77	28.88	27.77	28.33	33.33	45.00	

To gain increased accuracy in the estimation of error the data are divided into two parts, as a change in the effect of time on infected plant appears at about 1 hour. The large differences in numbers of infected plants for periods less than 1 hour and greater than 1 hour, cause these two parts of the analysis to have different standard errors. Part I is for 2, 5, and 15 minutes on the infected plant, and all times on the healthy plant, and Part II is for 1, 6, and 12 hours on the infected plant, and all times on the healthy plant. The analysis of variance is given in Table XIII.

TABLE XIII—ANALYSIS OF VARIANCE

NUMBER OF PLANTS INFECTED OUT OF THREE FOR TIMES OF APHID FEEDING ON INFECTED AND HEALTHY PLANTS

	Degrees of Freedom	Mean Square	
		Part I	Part II
Time on infected plants	2	8.505	1.373
Time on healthy plants	5	2.055	0.956
Interaction	10	0.806	0.818
Occasions	9	2.543	0.422
Error	153	0.684	0.401
Total	179		

The fraction representing the variance due to treatments is divided into time on infected plant, time on healthy plant, and their interaction, and it is convenient to examine the results separately in this order.

(a) *Time on Infected Plants*—Fig. 6 shows the variation in percentage infection for different times on infected plants, each point giving the results of 60 observations. In this graph, and in figs. 7, 8, and 9, the percentages are plotted against the logarithms of the numbers of minutes instead of the actual times, as this enables the positions of the points to be more evenly spaced.

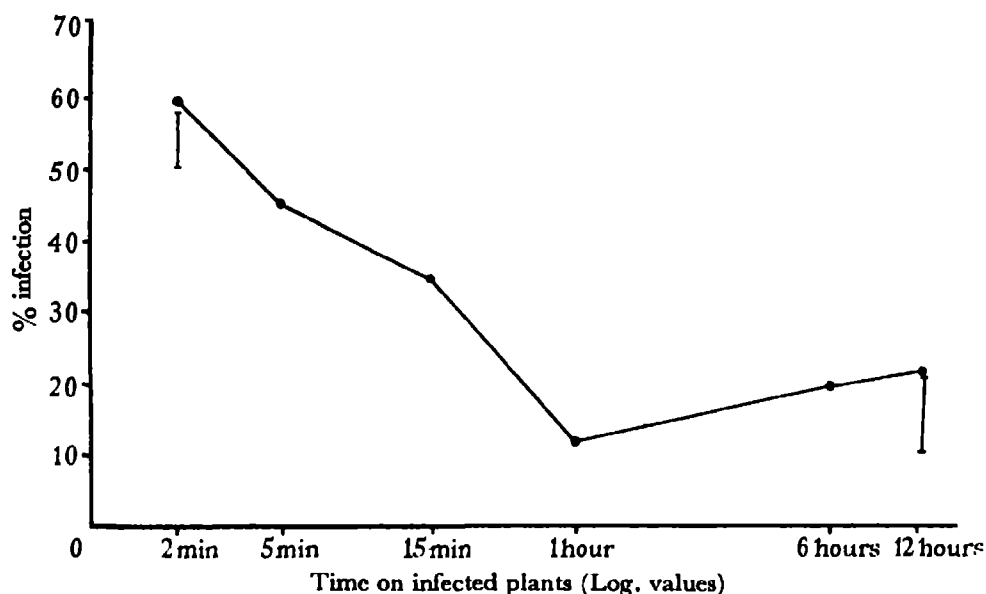


FIG. 6—Effect of varying times on the infected plant, for all times on the healthy plant. Vertical lines = significant differences (S.E. \times 3).

The high percentage infection from 2 and 5 minute feedings indicated in the preliminary experiment is here clearly demonstrated. From its highest point of 60% after only 2 minutes' feeding the percentage infection drops rapidly to a value of 11% for 1 hour's feeding, and then rises again very slowly to 21% for 12 hours' feeding. The form of the curve suggests that the rise may continue after 12 hours. The differences between all times from 2 minutes to 1 hour are significant, and also the rise from 1 hour to 12 hours. This can be seen from the analysis of variance, from the standard errors given in Table XII, and from the vertical lines representing significant differences given on fig. 6.

(b) *Time on Healthy Plants*—The two unbroken lines on fig. 7 represent the percentage infection for times on the healthy plant. The points on the upper curve represent the means of the values for 2, 5, and 15 minutes on the infected plant, those on the lower one being the means of the values for 1, 6, and 12 hours on the infected plant. Each point therefore represents the results of 30 observations.

The broken line represents the mean percentage infection for all times on infected plant. The significant differences are given as vertical lines beside each curve. Both curves have a significant regression of percentage infection on time as is shown by the analysis given in Table XIV :—

TABLE XIV

Time on healthy plant		Degrees of freedom	Variance	
			Part I	Part II
	Regression	1	5.762	2.333
	Remainder	4	1.087	0.611
	Error	153	0.684	0.401

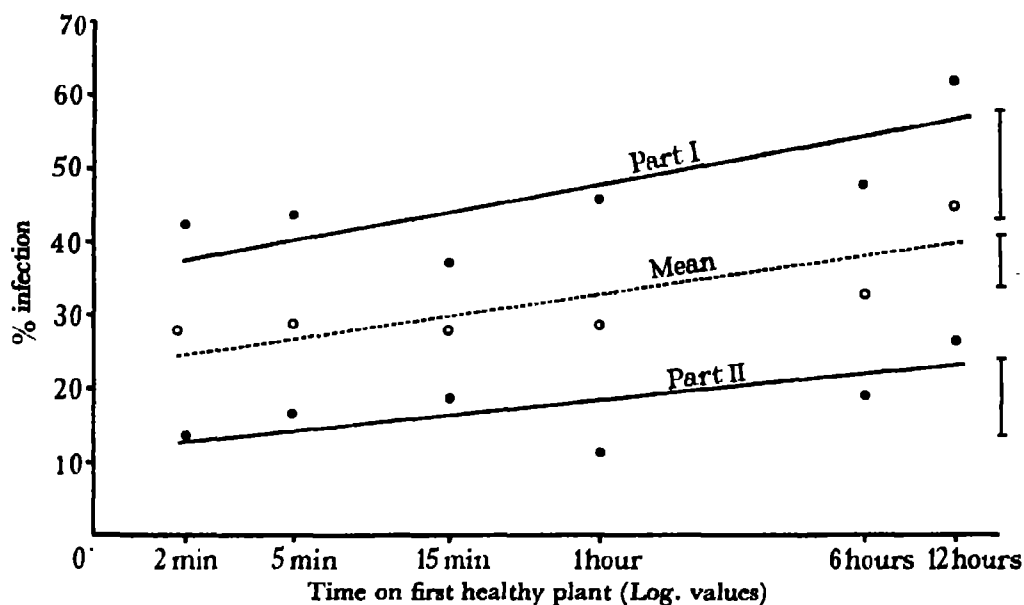


FIG. 7.—Effect of varying times on the healthy plant, for all times on the infected plant. Parts I and II of the analysis of variance and their means. Vertical lines = significant differences (S.E. \times 3).

There are apparent discrepancies at the ends corresponding to the short feeding times, but these are not significant. The discrepancy in the Part II curve is practically all in the line for 12 hours' feeding on the infected plant. This line shows high percentage values for the points corresponding to the shorter feeding periods, results which do not agree with those shown in figs. 4 and 5.

(c) *Interaction between (a) and (b)*—The analysis for Part I shows no significant interaction. For Part II the interaction is significant at the 5% level, but it is difficult to find any immediate explanation for this which would fit in with existing hypotheses. It is probably accounted for by the behaviour of the 12 hours' line which, as has already been pointed out, exhibits unexpectedly high values for the points corresponding to the shorter feeding periods.

There appear to be differences between the rates of increase of percentage infection with time on the healthy plant, corresponding to different feeding times on the infected plant. The regression coefficients are 0·21% for 1 hour's feeding, 0·63% for 6 hours', and 0·12% for 12 hours', but those for 1 hour's and 12 hours' feeding are not significant and they are not significantly different from the 6 hours' regression. On the other hand, two previous experiments, figs. 3 and 4, have shown a significant increase in percentage infection for time on the healthy plant after 12 hours' feeding on the infected plant, so that the present result is probably fortuitous.

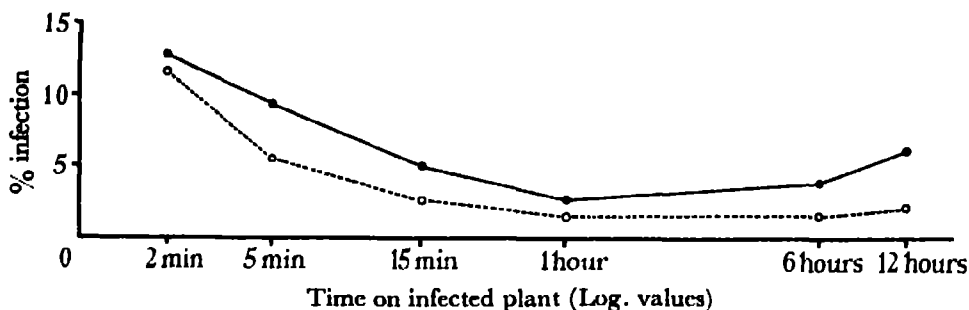


FIG. 8—Consecutive infections. Effect of varying times on the infected plant, for all times on the first healthy plant. • % of second plants infected; ○ % of second plants infected where first plants were also infected.

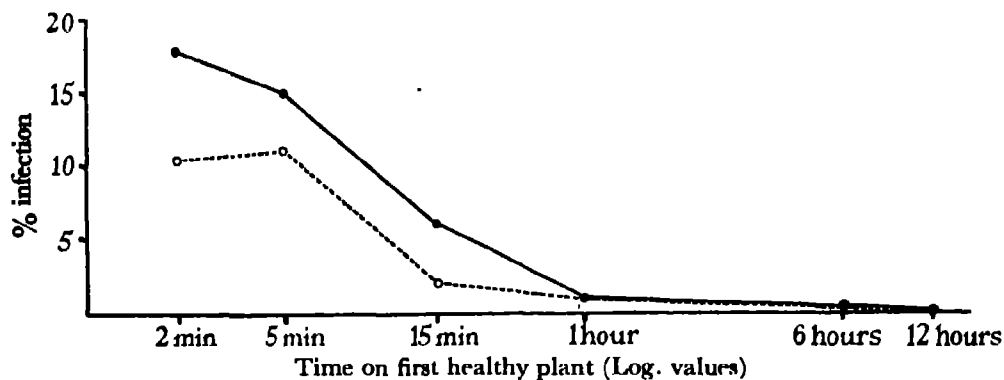


FIG. 9—Consecutive infections. Effect of varying times on the first healthy plant, for all times on the infected plant. • % of second plants infected; ○ % of second plants infected where the first plants were also infected.

(d) *Consecutive Infection*—Figs. 8 and 9 show the effect on percentage consecutive infection of times on infected and healthy plants respectively. The solid dots on each graph represent the total percentage of infection from the second feeding on healthy plants (B plants), and the open dots show the percentage of occasions on which both A and B were infected by the same aphid. The actual figures are given in Table XI.

It can be seen from fig. 8 that the curves for varying times on the infected plant have the same form as the corresponding curves for the A feedings (fig. 6), though

with smaller values, showing that there is a close relation between the total quantity of virus disseminated at the first feeding and the amount still active at the second feeding.

The infected and not infected B plants may be grouped in two classes, according to whether the corresponding A plant was infected or not :—

	B not infected	B infected	Total
A not infected	710	26	736
A infected	297	47	344
Total	1007	73	1080

From this arrangement of the data it can be seen that the proportion of second infections amongst aphids which secured a first infection (*i.e.*, $\frac{47}{344} = 0.137$) is greater than the proportion of second infections amongst aphids which did not secure a first infection (*i.e.*, $\frac{26}{736} = 0.035$). This difference is very highly significant and indicates that there is a real difference between aphids in their ability to transmit the virus. This may be due to variation in the capacity or opportunity for picking up infection from the infected plant, or else in the ability to transmit the infection when obtained.

There are two possible explanations, apart from differences in plant susceptibility, for the failure of certain aphids to infect the first plant on which they are fed though they succeed in infecting the second :—

(a) That they did not actually feed on the first plant.

(b) That there is variation in individual ability to infect, and that this is partly due to variation in the speed and efficiency with which the virus is transferred to the salivary glands for ejaculation into the plant.

It is not possible to distinguish between these, but consideration of the difference between the total numbers of infections obtained on B plants and those when both A and B were infected (shown as percentages in fig. 9) yields some information.

The numbers of infections of the second healthy plants for different times on the first healthy plant expressed as percentages of their own totals (as these give the best idea of the rate of decrease in percentage infection), are shown in Table XV for two kinds of infections.

TABLE XV

	2 min.	5 min.	15 min.	1 hr.	6 hr.	12 hr.
1. First healthy plant not infected. (Percentage of 26 infections)	50	23	26	0	0	0
2. First healthy plant infected. (Percentage of 47 infections)	40	44	9	4	2	0

If in Class 1 the aphid had always fed on the first healthy plant, the general trend of the figures might be expected to be the same in Class 1 as in Class 2, because in Class 2 it is known that the aphid must have fed on the first healthy plant.

If on the other hand certain of the aphids in Class 1 did not feed on the first healthy plant then the decrease from 2 minutes to 12 hours should be more rapid in Class 1 than in Class 2, because presumably a larger proportion of the aphids will not feed if they are only allowed to remain on the plant for a short time. There is, in fact, little difference in the rate of decrease, though that for Class 1 is if anything slightly steeper, but not sufficiently so to justify the assumption that all the difference is due to non-feeding on the first healthy plant.

The difference between the total number of B plants infected and the number of B plants infected where A was also infected, is smallest after 2 minutes' feeding on the infected plant (fig. 8), and we know from the curves for the A infections (fig. 6) that 2 minutes' feeding gives the highest number of infections on the first healthy plants. If failure to infect the A plant were due to non-feeding on the A plant the number of B infections where A was not infected, should be a constant proportion of the number of A infections, for all times on the infected plant, consequently the greatest number of B infections where A was not infected would be obtained for 2 minutes' feeding.

From these two considerations the influence of non-feeding on the first healthy plant does not appear to be important.

The consecutive infection curve for varying times on the healthy plant (fig. 9) is according to expectation. For 2 and 5 minute periods about 39% of the aphids which gave infection on the first plant, give consecutive infections, but the percentage falls off very rapidly with increasing feeding periods up to 15 minutes, and for longer periods only 3 consecutive infections were obtained out of 540 plants used. The chances of infection of a second healthy plant are therefore very low after more than 15 minutes on the first healthy plant. Within this period the number obtained is relatively high and it is possible that for the very short times more than one consecutive plant might be infected.

IV—DISCUSSION

The present work has shown that the amount of infection obtained in tobacco plants with the virus Hy. III, by means of the aphid *Myzus persicae*, varies in a regular manner according to external conditions. It has been found possible to estimate the probability of infection for different sets of conditions.

Many workers appear to have paid insufficient attention to the numbers of negative infections obtained in experiments. There has even been a tendency to attribute these "failures" to some arbitrarily selected factor or factors such as non-feeding of the aphids (even when 2 hours or more were allowed on suitable plants), or "variation in the ability of the aphids to cause infection". The assumption is, presumably, that if these factors could be properly controlled, 100% infection would result; but this attitude is due to misconception, and has caused the loss of much valuable information.

1—Seasonal Variation

As the virus agent, the insect vector, the healthy plant, and the infected plant are all concerned in the production of an infection, it is often difficult to distinguish which of them is affected by any particular set of conditions. Nor has it been possible to determine what seasonal climatic factor is responsible for seasonal variation. It seems, however, that of the four variables, the plants concerned are the most likely to be affected by climatic conditions, and of these intensity of illumination or length of day are presumably most important, as temperature and humidity in the glass-houses are partly controlled. The following evidence supports the hypothesis that seasonal variation is due to the effect of light on either the infected or healthy plants, or both, rather than on the aphids.

(1) Seasonal conditions visibly affect the degree of infection, because symptoms are severe, and often lethal in the winter months, but milder in the summer. It is possible that there is a high concentration of virus in the leaves during the winter, and this view is supported by the results of mechanical inoculations.

(2) Furthermore, SAMUEL, BEST, and BALD (1929) have found that pretreatment of healthy tobacco plants in subdued light increased their susceptibility to spotted wilt virus, and I have found that this is also true for Hy. III virus.

(3) The winter maximum percentage infection persisted even when the aphid cultures were receiving artificially increased and lengthened daylight, and therefore, if the effect is one of light, the aphids themselves are not concerned.

2—Numbers of Aphids

Given constant or equalized conditions the percentage infection increases in a regular manner with the number of aphids used per healthy plant. Also the infections are local and independent for each aphid and not the result of accumulations of sub-infective doses from different members of a group. This is of importance because it allows each aphid to be considered as an independent source of infection and makes possible the various hypotheses which are put forward to explain other effects.

Since 76% of single aphids are capable of causing infection in optimal conditions (see Table X), it is clear that the low numbers of infections which occur in other conditions, when the percentage infection may fall to zero, are not due to an inherent inability of some aphids to cause infection. STOREY (1928) has found for Streak disease of Maize that certain strains of the vector *Cicadulina mbila* possessed an inheritable incapacity for infection which was due (STOREY, 1933) to some peculiarity of the gut wall, but infectivity trials with several generations of different strains of *Myzus persicae* have given no evidence of consistent inability to infect. SMITH (1929) obtained similar results with potato virus Y. Thus, though consistently low percentage infection is obtained with single insect infections for many viruses (see SMITH, 1933; pp. 181–182), this does not necessarily indicate a fixed low standard of

efficiency in the vectors but is frequently due to fluctuations in their infective capacity which can be increased or decreased according to the conditions of the experiment.

3—Time on Infected Plant

The highest proportion of infection is obtained after 2 to 5 minutes' feeding on the infected plant. This might be explained in two ways. (a) More virus may be available to the aphid in those areas of the leaf which the stylets reach after two minutes' feeding ; (b) the drop in percentage infection with longer feeding periods may be caused by some effect on the virus produced within the insect.

a. *Availability of Virus in the Leaf*—BENNETT (1934) found that the virus of curly top of sugar-beet is confined to the phloem, and cannot be obtained from other tissues of the plant, and therefore it can only be carried by an insect which feeds upon the phloem. Similarly a connexion between other viruses, for example, that of potato leaf-roll, and the phloem tissues, have led to a general suggestion that the success of many vectors may be attributed to the fact of their being phloem feeders.

The rates of penetration of the stylets of *Myzus persicae* into tobacco leaves, similar to those used for the aphid infections, are being investigated and the results so far indicate that more than 5 minutes is required for the stylets to reach the phloem.* Since the percentage infection obtained after 2 minutes on the infected plant is so high it seems probable that Hy. III can be obtained from tissues other than the phloem, and that either the concentration of virus is higher in the superficial tissues than in the phloem or the subsequent fall in percentage infection is due to an independent action of the aphid on the virus.

b. *Effect on the Virus of Conditions Within the Aphid*—The aphids are starved for a few hours before feeding on the infected plant (p. 459) and this treatment may have had some effect on the percentage infection obtained from the shorter feeding periods on the infected plant.* Conceivably some of the virus is normally digested and starvation may cause the glandular cells of the stomach wall to enter a resting phase, so that the digestive enzymes are not secreted until a short time after food has entered the mid-gut. This hypothesis, however, assumes that dissemination of the virus into the blood stream occurs in the mid-gut, but the rapidity with which infection can be achieved indicates that dissemination begins as soon as the virus enters the alimentary canal, that is to say, through the extremely thin walls of the oesophagus. The writer has found that when the aphids are fed on intra-vital stains such as eosin, the walls of the oesophagus very quickly become brightly stained, whereas the mid-gut only receives a dilute solution and the dye rarely passes into the hind gut.

In HOGGAN's experiments (1933) the aphids were not subjected to preliminary starvation and no effect of feeding time on the infected plant was observed in the results. On the other hand, HOGGAN herself points out that her figures for the 5-minute feeding periods are not very reliable. In her experiments the assumption

* Since going to press both these suggestions have been confirmed.

that some of the aphids were not able to feed in the time allowed is justified, because no allowance was made for "penetration time" which in the conditions of the present experiments is about 5 minutes (p. 474). HOGGAN's figures show a slight drop for percentage infection after one hour's feeding, which might have been more obvious if single aphids had been used over a larger number of trials.

Another hypothesis which can be put forward to explain the high percentage of infection obtained by short feeding times on infected plants is that an antibody to the virus may be formed in the blood of the aphids. The formation of antibodies to various proteins has been described for a number of insects, and CHORINE (1931), using *Galleria mellonella*, finds that immunity can be acquired in from 2 to 7 hours, which agrees with the fact that the aphids appear to be least infective after from 1 to 6 hours' feeding (see fig. 6). The fact that infection was not completely suppressed indicates that many of the aphids do not acquire complete immunity, and it would have to be supposed that the antibody is produced at a lower rate than that at which the virus is imbibed. Also, to explain the gradual increase in percentage infection from 1 to 12 hours on the healthy plant, it might be assumed that a maximum rate of formation of antibody is reached, after which it is produced more slowly.

4—Time on Healthy Plant

The rise in percentage infection for feeding times on the healthy plants might also be explained on the antibody hypothesis, since MADSEN (1923) has shown that dilution of an antibody-antigen complex, before combination is fully completed, causes dissociation, leading to the production of free antigen, and the imbibing of fresh plant juice might dilute a partially formed antibody-virus complex.

The increase in percentage infection with time on healthy plant might also be explained, if in some aphids the virus circulates more slowly than in others, but this should not influence percentage infection after long periods on the infected plant. Another possibility is that the susceptibility of the plant tissues increases as the result of continuous injury by penetration of the stylets into increasing numbers of cells.

5—Consecutive Infection

The fact that aphids are capable of infecting two consecutive healthy plants without intermediate access to a source of infection makes it extremely doubtful that infection is caused by contamination from the outside of the stylets as HOGGAN (1933) and several other workers have suggested. This is in agreement with the conclusions from previous experiments on artificial feeding of *Myzus persicae* (HAMILTON, 1935).

There seems to be some disparity between the consecutive infection results for Hy. III and those of other workers for otherwise similar viruses. HOGGAN (1933) obtained no consecutive infection with cucumber mosaic after from 2 to 48 hours' feeding on the first healthy plant. DOOLITTLE and WALKER (1928) with the same virus state that none occurred after 10 to 20 minutes' feeding, but the actual figures are not

given. BENNETT (1932) using red raspberry mosaic and the aphid *Amphorophora rubi*, obtained no consecutive infection after 1 to 20 hours' feeding on the first healthy plant. Consecutive infection obtained after 20 hours he attributed to re-infection of the aphid with virus which had multiplied in the first plant. SMITH (1933) gives some evidence to show that *Myzus persicae* can infect a second healthy plant with crinkle Y virus after 24 hours' feeding on the first healthy plant, which may be a similar result to BENNETT's; but he gives no information about shorter feeding periods. Whether some of these viruses could be made to give true consecutive infection if shorter feeding periods and larger numbers of plants were used, remains to be proved. Preliminary experiments with Hy. III gave no consecutive infection after 2 hours' feeding on the first healthy plant (p. 19).

6—Note on Experimental Design

The accurate estimation of the probability of infection for any given conditions often required much larger numbers of plants than have generally been used. For those viruses in which single aphids give only a small percentage infection, this cannot be measured from 10 or 20 trials (see pp. 471, 472). Holme's local lesion method provides a quantitative measure of infection in a single leaf or plant when mechanical inoculation is used, but the local lesion technique has not been found to be practicable for insect infection with Hy. III. HOGGAN (1934) has succeeded in obtaining local necrotic lesions with individual aphid infections of Tobacco mosaic in a hybrid of *Nicotiana tabacum* \times *N. glutinosa*, and this technique promises to be of value in the further investigation of this virus. The starch lesions obtained in tobacco plants on which *Myzus persicae* infected with Hy. III virus have fed are often diffuse and very difficult to recognize, and either because of, or in addition to this, smaller numbers were obtained than would have been expected from whole plant infections in the same experimental conditions. This seems to indicate that not all aphids, which cause infection, form local lesions.

Where large numbers of plants have to be used, much economy of time and materials can be effected by employing an efficient experimental design, as in the factorial experiment described in § III, 5. The advantages of such complex experiments have been discussed by FISHER (1935).

Grateful acknowledgments are due to Mr. F. YATES for advice on the design of the experiments and for the statistical analysis of § III, 2; to Miss B. I. MITCHELL for assistance in the work; and to Mr. W. G. COCHRAN for criticism and advice.

V—SUMMARY

Experiments have been carried out in order to show the effect of various factors on the percentage of infection obtained with the virus Hy. III in tobacco using its insect vector, *Myzus persicae*.

Owing to differences in susceptibility to infection and concentration of the virus between leaves of different ages on the same plant, it is desirable to use leaves of corresponding ages for all aphid feedings in such experiments.

A maximum percentage infection was obtained during the winter months and a minimum during the summer months.

The percentage infection increases with the number of aphids used per plant, and the relation between the numbers of infections obtained for each aphid number indicates that the infections are local and independent.

The percentage infection increases with increased feeding time on the healthy plant, but there is no indication of a preliminary time period in which no infection is obtained.

The percentage infection decreases very rapidly with increasing time on the infected plant from 2 minutes to 1 hour. After 1 hour it increases slightly with further increase of the feeding periods.

The uncertainty as to whether or not aphids have fed on the trial leaves for the exact period allowed, was overcome by either "watched feedings" or by allowing an average "penetration time" of 5 minutes. "Penetration time" was found to be increased by decreasing relative humidity in the insectary.

Myzus persicae is capable of infecting two consecutive plants without intermediate access to a source of infection, but the number of second infections decreases rapidly with increasing time on the healthy plant, and is negligible after 1 hour.

For some aspects of the subject comparisons are made between Hy. III virus and others which appear to be of the same type. Suggestions are made as to the causes of some effects and the mechanisms of infection which are involved.

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